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# EFFECTS OF THE NEMATODE *ASCARIDIA LINEATA* (SCHNEIDER) ON GROWING CHICKENS*

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The importance of roundworms in the poultry industry has been problematical, as a high percentage of adult chickens harbor a small number of these parasites with apparently little injurious effect. Cobb (1896), however, held that the occurrence of these nematodes in numbers much above the average carried by the host in nature would cause serious results, and stated that the greatest loss was to be feared when young chickens became infested. Bradshaw (1907) considered worms as destructive to poultry as cholera and roup, and more dangerous because of the difficulty of diagnosis. In recent years, much of the unthriftiness of chickens has been attributed to these worms by poultry specialists. In the absence of experimental evidence on the effects of a nematode upon its host, the writers, in 1921, began a series of experiments on chickens using as a parasite the fowl nematode, *Ascaridia lineata* (Schneider).

Pure bred white leghorn chicks direct from the hatchery were placed at once in a tightly screened animal house with four well lighted, heated and ventilated experimental pens each 8 by 32 feet. That these quarters afforded ample space and suitable facilities was shown by Herrick, Ackert and Danheim (1923) who raised two generations of normal chickens in confinement, having kept many of the chickens confined continuously for three years.

The parasite (*A. lineata*) which is the large roundworm of chickens has a direct development. The thick-shelled eggs incubate outside the body of the host attaining the embryonated (coiled embryo) stage in about two weeks. Upon ingestion by the young chicken, the embryos are liberated in the small intestine where in two months they may grow to maturity.

The nematode eggs used in parasitizing these chicks were removed from well-developed females and cultured in Petri dishes containing distilled or tap water and a few drops of 2% formalin. The cultures

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were incubated at about 29° C. When rapid development was desired, they were aired daily by stirring the culture. At laboratory temperatures the eggs developed slowly and irregularly so unless retarded development was desired the eggs were incubated. The incubation period varied somewhat but in 10 to 14 days the eggs contained coiled motile embryos and were ready to be fed.

At the beginning of an experiment, the chickens were banded, kept off feed over night, weighed in the morning and separated into two lots of approximately equal weight, excluding any chickens that were known to be subnormal. The two lots were kept in adjacent pens under similar conditions, except that one lot was parasitized. Records of feedings, behavior, symptoms and weight were made regularly for each bird. In parasitizing, one of three methods was used: (1) filtering approximately equal volumes of the egg culture and feeding the filter paper and eggs to the separate chickens (chicks one month or more of age), (2) feeding small quantities of wet mash into which the eggs had been mixed, and (3) with aid of pipette, placing in the gullet a liquid starch paste into which the worm eggs had been stirred. This method was developed by Naomi B. Zimmerman. At autopsy the intestine was removed immediately after killing, opened and its entire contents scraped into a receptacle containing warm tap water from which the worms were counted.

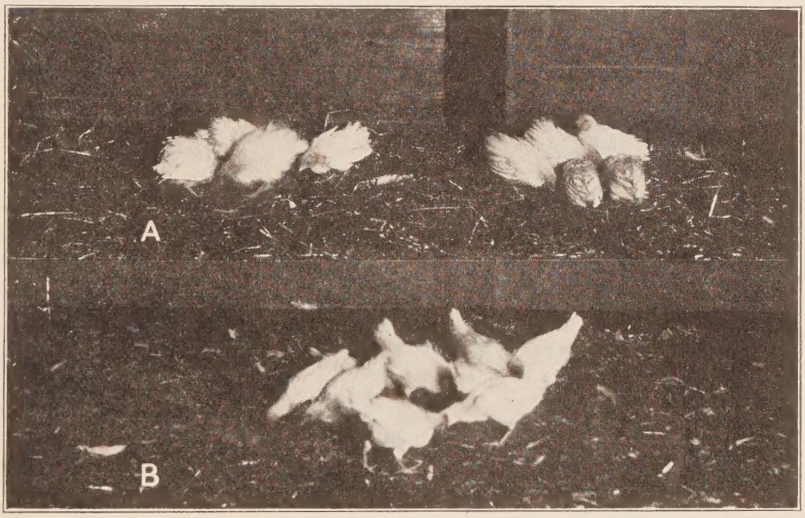
## EXPERIMENTAL RESULTS

In these experiments 411 chickens were used, approximately one-half of which were parasitized with the nematode, *A. lineata,* the other half being used as controls. The first visible effect of parasitism was sluggishness which appeared in eight to ten days after the first feeding of embryonated eggs, the time of the onset depending upon the degree of infestation and the age and vitality of the bird. As a rule, the younger the chick, the more marked was the effect. Young chicks two months or less of age besides being sluggish, showed loss of appetite. By the twelfth day, drooping wings and ruffled feathers (text fig. *A*) began to appear, the controls (text fig. *B*) showing none of these symptoms. Each day the parasitized chicks grew weaker and by the fourteenth to the sixteenth day, several of the chicks assumed the position shown in Plate 1*A* and died of parasitism.

Marked internal effects due to parasitism were plainly visible at the autopsies of the chicks. Greatly retarded muscular and bone development, characteristic effects, are seen in Plate 1, *C* and *D* which shows the breasts of the chicks pictured in *A* and *B*. That of the parasitized chick (*C*) was almost devoid of muscle. Body fat, present in the typical controls (*D*), was lacking in parasitized birds. The testes of the latter were proportionately smaller and the ureters frequently distended with urates.

Chicks six to eight weeks of age when first parasitized were less affected by the parasitism. They usually retained more strength, showing the drooping wings and ruffled feathers to a lesser degree and for a shorter period of time. However, many of them were so devitalized by the parasitism and accompanying factors as to be unable to recover (Plate 2*A*).

*Experiment I.*—One of the most definite measures of the effect of this parasite upon the chickens was that of body weight. In this experiment, approximately 1,000 eggs of *A. lineata* were given to each of 27 chickens 49 days of age, averaging 183 gm. in weight. As controls a similar number of chickens from the same hatch and of the same average




Text figure.—*A.* Photograph of parasitized chicks; thirteenth day of parasitism; fed embryonated eggs of *A. lineata* at age of 41 days; note the drooping wings and ruffled feathers. *B.* Control chicks from same hatch. Note larger size and smoother feathers.

weight was used. These two groups were kept under the same conditions except that of parasitism, the effects of which began to appear during the second week. Subsequent feedings of embryonated eggs were made to the parasitized group when the chicks were 60, 79, 141, 145 and 196 days of age, respectively. At the end of two weeks both lots were taken off feed over night and weighed.* The parasitized group made an average gain of 92.3 gm. per chick and the controls 119 gm., or a difference of 26.6 gm. Since this difference is 7.17 times (as much as four

* Tables giving the weights and gains in weight made by each chicken in the various experiments and the respective numbers of worms are available for any investigator who may wish to examine them.

times) its probable error, the difference in gain between the parasitized and control chickens during this period was significant.†

During the second two-week period, the infested chicks were still suffering from the parasitism, especially during the first half of it, but toward the end of the fourth week many of the chicks showed improvement. Weights made at the close of this week showed an average gain of 257 gm. for the parasitized group and 307 gm. for the controls, or a

TABLE 1.—*Summary of the Gains in Weight made by the Parasitized and Control Chickens in the Various Experiments*

| Periods, Weeks | Parasitized Chicks | | Control Chicks | | Difference Gain (Grams) in Favor of Controls |
|---|---|---|---|---|---|
| | Average Gain, Grams | Standard Deviation | Average Gain, Grams | Standard Deviation | |
| | | | Experiment 1 | | |
| 0 - 2 | 92.3 ± 2.4 | 18.55 ± 1.7 | 119. ± 2.85 | 22. ± 2.02 | 26.6 ± 3.7 |
| 2 - 4 | 257.0 ± 8.3 | 64.00 ± 5.89 | 307. ± 9.99 | 77. ± 7.06 | 50.0 ± 12.98 |
| 4 - 8 | 302.0 ± 6.47 | 48.00 ± 4.41 | 291. ± 13.00 | 97. ± 8.91 | Parasitized gained more than controls |
| | | | Experiment II | | |
| 0 - 2 | 41. ± 1.30 | 15.06 ± 0.92 | 67. ± 1.09 | 12.6 ± 0.76 | 26. ± 1.5 |
| 2 - 4 | 133. ± 2.81 | 29.79 ± 1.99 | 164. ± 2.6 | 30.0 ± 1.90 | 31. ± 3.74 |
| 4 - 8 | 300. ± 5.8 | 62.00 ± 4.14 | 265. ± 4.21 | 48.0 ± 2.98 | Parasitized gained more than controls |
| | | | Experiment III | | |
| 0 - 2 | 38.43 ± 2.65 | 21.50 ± 1.87 | 61.6 ± 2.33 | 19.0 ± 1.65 | 23.17 ± 3.53 |
| 2 - 4 | 59.75 ± 1.66 | 8.40 ± 1.15 | 88.2 ± 4.17 | 29.1 ± 2.96 | 28.45 ± 4.48 |
| 4 - 8 | 261.00 ± 15.06 | 67.00 ± 10.65 | 352.0 ± 9.93 | 59.2 ± 6.84 | 91.00 ± 18.00 |
| | | | Experiment IV | | |
| 0 -17 (days) | 15. ± 5.11 | 25.09 ± 3.6 | 88. ± 5.32 | 19.23 ± 4.36 | 73. ± 7.34 |
| | | | Experiment V | | |
| 0 - 1 | 33.28 ± 1.98 | 11.00 ± 1.25 | 33.26 ± 2.7 | 15.51 ± 1.71 | 2.98 ± 3.34 |
| 1 - 2 | 34.80 ± 1.29 | 7.21 ± .91 | 48.10 ± 2.06 | 11.83 ± 1.45 | 13.30 ± 2.43 |
| 2 - 3 | 27.00 ± 2.14 | 11.74 ± 1.49 | 39.00 ± 2.57 | 14.73 ± 1.81 | 12.00 ± 3.31 |
| | | | Experiment VI | | |
| 0 - 1 | 7.50 ± .48 | 5.00 ± 0.33 | 11.00 ± 0.93 | 9.70 ± .66 | 3.50 ± 1.00 |
| 1 - 2 | 26.96 ± 1.47 | 15.33 ± 1.04 | 38.59 ± 1.31 | 13.58 ± .92 | 11.63 ± 1.97 |
| 2 - 3 | 20.45 ± 2.20 | 22.63 ± 1.55 | 32.06 ± 2.59 | 26.90 ± 1.83 | 11.61 ± 3.39 |
| | | | Experiment VII | | |
| 0 - 1 | 18.35 | | 6.53 | | Parasitized gained more than controls |
| 1 - 2 | 49.92 ± 3.46 | 19.18 ± 2.44 | 81.60 ± 4.39 | 25.19 ± 3.1 | 31.68 ± 5.58 |
| 2 - 3 | 23.80 ± 2.79 | 15.50 ± 1.97 | 64.30 ± 6.05 | 34.70 ± 4.27 | 40.50 ± 6.66 |

difference of 50 gm. which was probably significant. During the sixth week there were further signs of improvement in the parasitized group. Drooping wings were less in evidence and most cases of extreme weakness were disappearing. The seventh week brought more improvement, especially in plumage and appearance. During the eighth week practically all of the chickens of the parasitized group were in a vigorous condition, and when weighed showed the surprising average gain of

† The writers wish to acknowledge indebtedness to Dr. W. H. Andrews and Dr. H. L. Ibsen for counsel in the biometrical treatment of the data.

302 gm., to 291 gm. for the controls. The increased gain during this period had brought the average weight of the parasitized group up to 834 gm. as compared with 854 gm. for the controls, and by the end of the next month the former group appeared as thrifty and completely feathered as the latter.

Since it was desired to study the effect of parasitism upon egg production, these chickens at the age of 141 days were again given a heavy feeding (approximately 2,000) of embryonated eggs of the parasite. Four days later another feeding was administered. But careful observations failed to reveal any effect upon the appearance or behavior of the chickens at any time during the succeeding month. Another large feeding of parasite eggs made when the chickens were 196 days old likewise failed to affect the appearance or behavior of the birds.

When the chickens were 34 weeks old, those numbered 471 to 516 were autopsied. Half of these parasitized birds had lost all of their nematodes, and the remaining ones had 1, 7, 4, 6, 3, and 2 worms, respectively. None of the controls was infested. The remaining chickens in these groups were autopsied at the age of 45 weeks. Three of the parasitized group were without worms; the other 12 had the following respective numbers of worms: 1, 26, 7, 28, 5, 3, 16, 9, 5, 4, 1 and 7. The cases of chickens 18 and 20 are noteworthy as both were vigorous birds weighing 930 and 907 gm., considerably above the average, and both made large gains during the preceding two-week period in spite of an infestation of 26 and 28 adult worms, respectively. The results of this experiment show that parasitism had a significant effect upon the weights of the chickens during the first two weeks. During the second two-week period the difference in gain was probably significant, but during the succeeding month the parasitized group gained more than did the controls, almost equaling the latter's average weight.

*Experiment II.*—Having tested the effects of the nematodes on chickens first parasitized at the age of seven weeks, it was decided to test the effect on chicks two weeks of age. For this experiment 122 chicks were isolated and divided into two equal lots, the one to be parasitized averaging 65 gm. and the controls 61 gm. Approximately 2,000 embryonated eggs of *A. lineata,* mixed into a wet mash, were given to each chick of the parasitized group when they were 14 days of age. Subsequent feedings of worm eggs were made when the chicks were 22, 45, 53, 59, 69, 89 and 102 days old, respectively. Symptoms of parasitism began to appear on the ninth day, and one chick died on the eleventh day. At the end of two weeks, the parasitized group had made an average gain of 41 gm. and the controls of 67 gm., there being an average difference of 26 gm. which was very significant (Table 1). As in the previous experiment, after the onset of the symptoms the

severity of the parasitism increased. Besides one chick dying on the eleventh day, six died on the fourteenth, one on the fifteenth and one on the sixteenth day, making a total of nine for the group or a mortality of 14.9%. During this period one of the controls died, a mortality of less than 2%.

At the end of four weeks, the remaining chicks of the parasitized group had made an average gain of 133 gm. and the controls, 164 gm. Here the difference of 31 gm. was significant. During this period the condition of the surviving parasitized chicks improved, indicating that they were slowly overcoming the effects of the infestation. After the sixteenth day there were no deaths, either among the parasitized or the control group. Weights were next made after a period of four weeks, or at the close of the eighth week of the experiment. During this time, the parasitized chicks made an average gain of 300 gm. and the controls, 265 gm., thus exceeding the controls' gain in weight and nearly equaling them in actual weight, the parasitized group now averaging 544 gm. and controls 557 gm.

Concerning the presence of worms, autopsies were made at different periods, some when chicks died (nine at approximately four weeks, and two at 10 weeks of age) and others when they were killed. A few were autopsied when the chickens were eight weeks old, several when they were 20, but most of the parasitized ones when they were 36 weeks old. The nine chicks that died during the second and third weeks were all heavily infested, having 960, 1,780, 1,160, 1,650, 920, 1,640, 1,190, 1,440 and 1,850 young worms, respectively. A tenth chick, No. 584 (Pl. 1*A*), killed for comparison with a typical control, No. 649 (Pl. 1*B*), contained 1,240 young worms. Only three of the remaining 51 parasitized chicks were infested when they were autopsied. Chicken 566 autopsied at the age of 36 weeks had one worm. Chicken 604 examined when 36 weeks of age had 44 *A. lineata*, and chicken 610 autopsied at 21 weeks of age had a single worm. None of the controls was infested. One control chicken, 617, subnormal in weight, died of unknown cause. From this experiment it is seen that the worms had significant effects on the gains of the chickens both at the end of two weeks and four weeks of parasitism; that the effects were most severe during the first two weeks; that the weights were markedly affected through the second two-week period, but that during the second month of parasitism the deleterious effects rapidly disappeared as judged by the appearance and behavior of the chickens and by their accelerated rate of gain, which exceeded that of the controls.

*Experiment III.*—The object of this experiment was to test the effect of the worms on chicks three weeks of age. Sixty chicks from the same hatch were separated into two lots of 30 each, the group to be parasitized

averaging 76.8 gm. and the controls 80.9 gm. When 21 days of age the former group was parasitized by being fed approximately 2,000 embryonated eggs of *A. lineata* mixed into a wet mash. Additional feedings of embryonated eggs were made when the chicks were 23, 26, 29, 34 and 52 days old, respectively. Marked symptoms of parasitism began to appear on the ninth day, and on the tenth day one chick died, having an infestation of 1,740 young worms. Several chicks were greatly weakened by the parasitism and there was soon a heavy mortality, three dying on the eleventh day, two on the thirteenth, and 11 on the fourteenth day, all of these chicks having from 700 to 1,950 young worms in their intestines, whereas among the controls there were no deaths. Weights made at the close of the second week showed that the parasitized chicks had made an average gain of 38.43 gm. and the controls 61.6 gm., or a significant difference of 23.17 gm.

Marked symptoms of infestations continued throughout the next two-week period and on the twenty-eighth day there was a mortality of three more chicks which harbored 65, 60 and 225 worms, respectively. Weights then made showed that the parasitized group had made an average gain of 59.75 gm. and the controls 88.2 gm., a difference of 28.45 gm. which was significant. During the following month the nine surviving parasitized chicks improved in general appearance but were unable to keep pace with the rapidly growing controls which in the four-week period made an average gain of 352 gm. as compared with 261 gm. for the parasitized group or a significant difference of 91 gm. in favor of the controls. The experiment was terminated at this time (close of the eighth week of parasitism; age of chicks 11 weeks). The autopsies showed that of the nine surviving parasitized chicks, six had eliminated all of their nematodes and that the three remaining ones had only two, four and twenty-two worms, respectively. The evidence from this experiment indicates that heavy infestations of roundworms in young chicks three weeks of age are very detrimental to their size and vigor, that the most critical time during the parasitism is the fourteenth day and that a heavy mortality (more than 68%) may occur. The evidence indicates that repeated feedings of worm eggs to chickens of this age during one month may limit the growth of the chickens throughout the first two months of parasitism. The results also indicate that as the chicks grow older they have increasing power of eliminating worms from their intestines.

*Experiment IV.*—In Experiment I, which was started with chicks 49 days of age and extended over a rather long period during which most of the worms had been lost, it was uncertain whether or not all of the fowls had been infested. Consequently, a test was made on birds of that age with a view of examining them at an earlier period. Eleven

chicks averaging 256 gm. in weight were each given approximately 2,000 embryonated eggs of *A. lineata;* and six chicks from the same hatch, averaging 238 gm. were used as controls. One of the chicks died on the fifteenth day and two on the seventeenth day, all three of them being heavily infested. As many of the remaining parasitized chicks were in a greatly weakened condition, the experiment was terminated on the seventeenth day. On weighing, it was found that only four of the infested group had gained in weight, the average gain being 15 gm. per chick, whereas, the control group made an average gain of 88 gm. per bird or the very significant difference of 73 gm.

At autopsy 100% of the chicks were infested, the approximate respective numbers of young nematodes being 27, 300, 310, 400, 500, 630, 800, 800, 900, 900 and 900. Thus, only one chick, a vigorous bird, succeeded in eliminating most of the worms from its intestine. Among the control group none of the chicks was infested. The mortality of three chicks in the parasitized group was doubtless kept to that figure by virtue of terminating the experiment, several of the surviving chicks at that time being greatly weakened and in a very critical condition. In the control group there were no fatalities and no weak chickens. From this experiment it was found that chicks 49 days of age fed approximately 2,000 worm eggs all became infested with young worms. The results also showed marked effects of parasitism by loss of weight (six chickens), by a mortality of 27% and by failure to make gains comparable with those of the controls.

*Experiment V.*—In this experiment the test on the effects of the nematodes was begun when the chicks were approximately one month (28 days) old. As constant results had been obtained on the effects of parasitism with heavy infestations, it seemed desirable to ascertain the effects from much smaller feedings. Instead of large repeated feedings, one administration of about 500 eggs was made to each bird. During this and the subsequent experiments, the feedings were administered with the aid of a pipette, the eggs having been stirred into a starch mixture. The group parasitized numbered 14 chicks and averaged 114 gm. in weight. Fifteen chicks averaging 122 gm., from the same hatch, were used as controls. Weekly weights were taken. At the close of the first week there was but little difference between the gains of the two groups. During the second week the parasitized group gained 34.8 gm. and the controls, 48.1 gm. making a difference of 13.3 gm. which was significant (Table 1). In this period the chicks showed characteristic symptoms of parasitism. At the close of another week the experiment was terminated. An average gain of 27 gm. was made by the parasitized group and 39 gm. by the controls, a difference which was probably significant.

At autopsy all but one of the parasitized chicks were infested, the respective numbers of nematodes being 4, 7, 8, 8, 10, 13, 17, 17, 18, 25, 25, 29 and 42, or an average of 15.9 worms per bird. None of the control chicks was infested. In this experiment it is seen that even though a smaller number and a single feeding of eggs were given, the worms had a significant effect upon the chickens during the second week of parasitism. By the end of the third week, however, the parasitized group showed sufficient improvement (probably from elimination of worms) to make the difference in gain of doubtful significance. As in Experiment IV, the examination of the chicks after a short period of parasitism showed a high percentage (92.8) of infestations.

*Experiment VI.*—For further testing the effect of a rather light infestation, chicks of the age of three and one-half weeks (25 days) were chosen. As in Experiment V, approximately 500 embryonated eggs of the parasite were given to each of 50 birds of a group which averaged 86 gm. The 49 controls had the same average weight. During the first week some effects of parasitism could be detected, as this group gained an average of 7.5 gm. and the controls 11 gm (Table 1). Distinct evidences of parasitism began to appear about the tenth day and on the thirteenth day one chick died, having harbored 367 young worms. At the close of the second week many of the parasitized chicks were visibly affected, the average gain for this group being 26.96 gm. and for the controls, 38.59 gm., or a significant difference of 11.63 gm.

Early in the third week most of the chicks in the parasitized group were still plainly affected and on the seventeenth day another chick died, having an infestation of 394 worms. By the end of the third week when the experiment was terminated, the parasitized group had made an average gain of 20.45 gm. and the controls 32.06 gm., or a difference of 11.61 gm. which was probably significant (Table 1). At autopsy it was found that two chicks were without worms. All the rest of the parasitized group were infested with the following respective numbers of young nematodes: 1, 1, 1, 1, 1, 3, 3, 3, 3, 4, 4, 4, 5, 5, 5, 6, 7, 7, 7, 8, 8, 8, 9, 10, 10, 10, 11, 11, 11, 11, 14, 14, 15, 17, 17, 22, 23, 25, 27, 37, 39, 45, 86, 93, 110 and 111. These infestations together with the two of 367 and 394 worms each give a range of infestation of from one to 394 worms or an average of 34 worms per bird. These results show that a single smaller feeding of *A. lineata* eggs had significant effects upon the weights of young chickens at the end of two weeks of infestation, and probably also during the first and third weeks. The results likewise show that examination of the chickens after three weeks of parasitism yielded a high (96) per cent of infestation.

*Experiment VII.*—Tests on the effects of parasitism having been made on chicks of various ages up to seven weeks, it was now decided

to make a test on chicks two months old (61 days). Fourteen chicks averaging 281 gm. were given one feeding of approximately 500 eggs. The 15 controls had an average weight of 273 gm. Throughout the first week all chicks appeared normal, the parasitized group outgaining the controls. Toward the end of the second week much sluggishness appeared among the parasitized group whose average gain for the week was 49.92 gm. while that of the control group was 81.6 gm. This difference of 31.68 gm. was significant (Table 1). During the third week, the parasitized group was plainly affected, their average gain for this period being only 23.8 gm. as compared with 64.3 gm. for the controls, or a difference of 40.5 gm. which was likewise significant.

At the end of three weeks all birds were autopsied. Two of the parasitized group were without worms; the rest had the following respective numbers of young worms: 2, 2, 6, 6, 6, 8, 8, 11, 18, 33, 50, and 65, or an average infestation of 15.3 young worms per chicken. From the results of this experiment, it is seen that chickens two months of age given a single feeding of 500 eggs were significantly affected during the second and third weeks of parasitism. The results showed that at the end of three weeks only two chicks were able to eliminate all of their worms, thus leaving 12 of the 14 birds, or a percentage of 85.7, of infested chicks.

## DISCUSSION OF RESULTS

The effects of the nematodes were most marked during the first month, especially from the tenth to the seventeenth day when 31 of the 34 mortalities occurred. This period corresponds exactly with the time during which the young worms have been found with their anterior ends buried deeply in the intestinal wall (Ackert, 1923) at the expense of some of Brunner's (Lieberkühn's) glands. During this period, also, erythrocytes were found in abundance in the feces, showing that there was considerable loss of blood. The injury to the intestinal wall, with accompanying possibilities of bacterial infection, the loss of blood, the presence of metabolic wastes from the worms, and the effects resulting from impaired appetites seem sufficient to explain the observed symptoms of parasitism.

As soon as the young worms withdraw from the intestinal wall and become free in the lumen of the intestine (from about the eighteenth day of parasitism on) the greatest severity of the infestation begins to pass. Elimination of the young worms from the intestine, evidently in progress from the time of parasitizing, proceeds more rapidly at this time and in the course of a few weeks usually results in the loss of the great majority of the worms and in a gradual recuperation of the surviving parasitized chicks. Thus in Experiment III, where approximately 2000 eggs were given to each chick, the 17 chicks that died during the first 14 days were infested with from 700 to 1950 young

worms, whereas the three chicks that died on the 28th day had 60, 65, and 225 worms, respectively. Also in Experiment VI, where the chicks were each given 500 embryonated eggs, the two chicks that died during the first 14 days had 367 and 394 worms, respectively, while on the twenty-first day (termination of experiment) two of the chicks were without parasites and the remainder had from one to 111 young worms. Further evidence supporting this point is afforded by the percentage of infestations of chickens examined after about three weeks as compared with that of chickens autopsied several weeks later. At the close of Experiments IV, V, VI, and VII which were terminated after about three weeks of parasitism, the respective percentages of infestation were 100, 92.8, 96, and 85.7, whereas those in Experiments I, II, and III terminated in from eight to 45 weeks were 66.7, 0.05, and 33.4, or an average percentage of 93.6 for the former and 33.4 for the latter. With the loss of most of the worms, the appetite returns and in the course of several days many of the birds become voracious, take quantities of food and thus for a time gain more rapidly in weight than do the controls. This change in behavior usually occurs during the fourth and fifth weeks of parasitism. Occasionally, chickens are unable either to eliminate most of the worms or to increase their vigor and are thus permanently disabled (Pl. 2*A*), such failure in nature probably being due to nutritional deficiencies, complication with other parasites or with pathogenic bacteria, or to inherited susceptibility.

Definite resistance to the nematode, *A. lineata*, was manifested in 1921 by chickens approximately three months of age, as subsequent feedings of embryonated eggs given after the chickens were 79 days old made no visible effect upon the birds (Experiment I). Whether this resistance was due to the previous infestation or to the age of the chickens was not determined.* That the resistance to the nematodes may have been due to age of the chickens was shown by Herrick (1925) who experimentally infested chickens of different ages with these nematodes and found that as growing chickens became older their resistance to the growth of the worms increased. In the present experiments, very young chicks heavily parasitized showed little resistance to the nematodes. For example, 15 chicks 11 days of age were isolated from a brood and given approximately 2000 embryonated eggs to furnish a demonstration of parasitism. Characteristic symptoms began to appear on the ninth day and all chicks were dead by the close of the sixteenth day. None of the remaining chicks of the brood died or became sick during this period.

As stated, it was desired to ascertain if parasitism with the large roundworm, *A. lineata*, had any effect upon the egg production of the

* Results of tests by Ackert, from 1923 to date, on this point will soon appear.

chickens. The pullets began to lay when they were about five and two-thirds months of age. In Experiment I, twelve of the thirteen pullets in the parasitized group were isolated, and twelve (random selection) of the 16 pullets from the control group were likewise isolated. When it was determined that all pullets of both groups were laying, egg records were made for each group. During five and one-half months, the parasitized group layed 720 eggs, and the controls, 660 eggs, no detrimental effects from parasitism appearing.

In Experiment II, 22 of the surviving parasitized pullets were isolated and 22 of the controls selected at random. Here again all pullets were trapnested until it was found that all were laying. In the 38 days that followed, the parasitized group layed 66 more eggs than did the controls. During the next two and one-half months, the parasitized group remained in the lead, laying 438 eggs, while the controls were laying 358 eggs. Over a longer period the difference would probably have been negligible, but the results of these tests fail to indicate that the egg production was reduced by the parasitism, either in numbers or weight of the eggs. In the second experiment the larger production of eggs by the parasitized pullets might be attributed to the loss of the inferior birds by death, but this would not account for the larger number of eggs from the parasitized group of Experiment I, as no deaths occurred in it. It seems probable that the increased appetite and more rapid gains in weight during the later periods might explain the increased number of eggs. With the exception of pullets 18, 20, and 24 (Experiment I) with 26, 28, and 16 worms, respectively, and pullet 604 (Experiment II) with 44 worms, there is no evidence that the laying pullets were sufficiently infested during the laying period to interfere with their normal growth and development, the other pullets probably having lost all, or nearly all, of their worms several weeks before laying time. As the pullets were not trapnested throughout the tests, the individual laying records for the birds are not known, but it is obvious that under the conditions of these experiments, early heavy parasitism of young chicks does not reduce the egg-laying ability of the surving birds.

Studies in progress on possible effects of parasitism on the blood of chickens will be published in a separate paper. Other effects of this nematode *(A. lineata)* on chickens now include significant reductions in size of the thymus glands (Ackert, 1924) and lessened amounts of sugar in the blood (Ackert and Titus, 1924).

## SUMMARY

1. Effects of the nematode, *Ascaridia lineata* (Schneider) on its host, the domestic chicken, were studied in experiments involving 411 chickens raised in confinement.

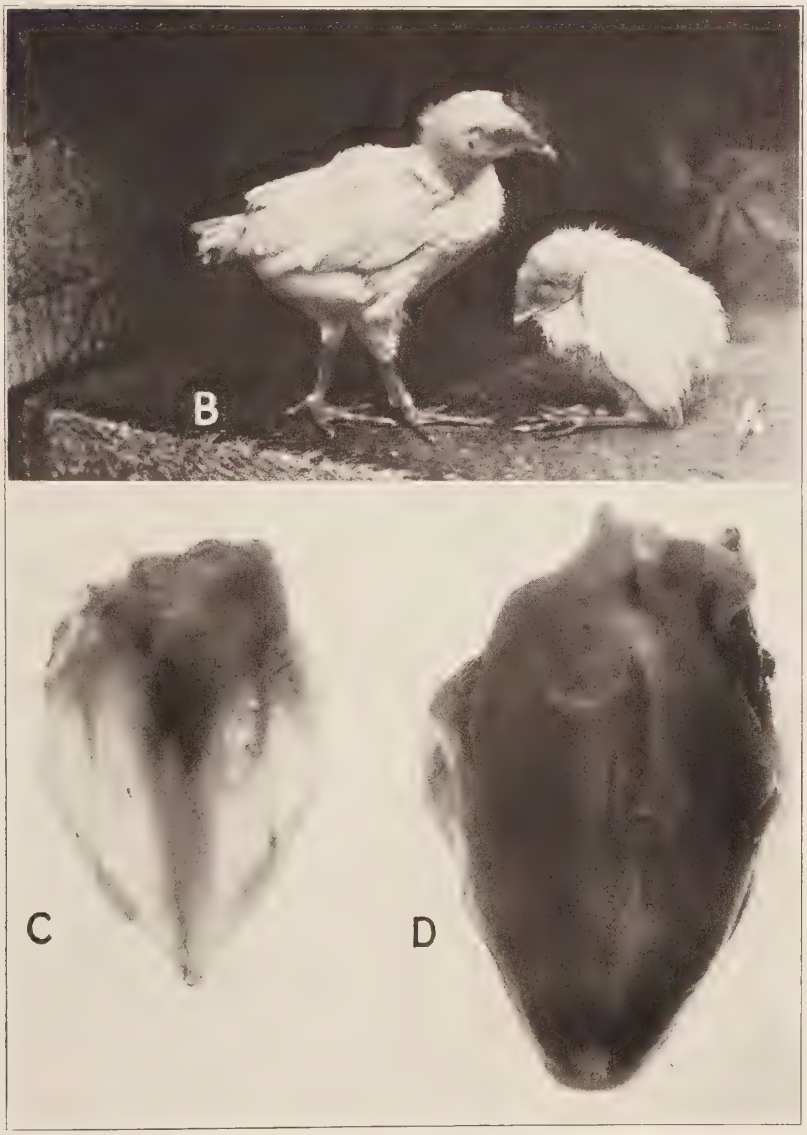



PLATE I

*A*. Photograph of parasitized chick (No. 584, Exper. II); fourteenth day of parasitism; fed embryonated eggs at age of fourteen days; typical of fatal cases; heavily infested. *B*. Control chick (No. 649, Exper. II) from the same hatch; eight grams heavier at beginning of experiment, 68 grams heavier two weeks later when picture was taken. *C*. Showing photograph of breast of chick 584 parasitized and of *(D)* breast of control chick 649.


PLATE II

*A*. Parasitized chicken unable to recover from parasitism. *B*. Control from same hatch; age nearly four months.

2. The symptoms which were most pronounced in young chickens during the first three weeks of parasitism were: sluggishness, loss of appetite, ruffled feathers, drooping wings, loss of blood and of body weight, retarded muscular and osteological development, urates in the ureters, and increased mortality.

3. The observed effects of this nematode upon its host are attributed to injury by the parasite to the intestinal wall, loss of blood, probable bacterial infection, absorption of metabolic wastes from the worms and partial inanition resulting from lost appetite.

4. Elimination of nematodes from the intestine seemed to occur continuously from the time of hatching until most of the worms were lost, but the rate of loss was accelerated after about the eighteenth day.

5. Chicks that survived three weeks of parasitism usually recovered, gradually gaining in strength and improving in plumage until they eventually approached, and sometimes exceeded the controls in vigor, appearance, and performance.

6. Chickens previously parasitized manifested a definite resistance to the nematodes by the time they were approximately three months old; but such chickens were significantly affected by subsequent parasitism from eggs given when the chicks were less than three months of age.

7. Two tests on effects of *A. lineata* on egg production of chickens failed to show any retardation or inhibition of this process.

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# FURTHER STUDIES ON THE LONGEVITY OF THE EGGS OF *ASCARIS LUMBRICOIDES* AND *A. SUUM* *

H. W. Brown

That the eggs of *Ascaris lumbricoides* remain alive and infective for long periods of time under laboratory conditions of storage is well known. Davaine (1863) found that the eggs of this parasite were infective after five years' storage. Morris (1911) noted motile embryos in eggs after more than two years' storage in a dilute formalin solution and Epstein (1892) produced an infestation with eggs a year old. More recently Martin (1926) reported that the eggs of *A. suum* stored for four years in a moist condition at temperatures ranging from –5° C. to 10°C. developed to the embryonated stage when given an optimum temperature. The conditions of these experiments, however, are not similar to those found in nature and the results should be taken with reserve in judging the longevity and viability of Ascaris eggs under field conditions. A number of experiments upon the longevity of Ascaris eggs under conditions more closely approximating those found in nature have been reported and although none of them extended longer than a year they also indicate a rather high resistance of these eggs to external conditions. Thus Baillet (1866) in France, found that the embryonated eggs of *A. suum* remained viable after 12 months' exposure to the heat of summer and the cold in winter. Yoshida (1920) reported experiments in which eggs were put on the ground and under a layer of soil and left through the winter months in Osaka, Japan. Their infectivity was then proved by the migration of the larvae through guinea-pigs. Martin (1922) likewise reported that the eggs of *A. suum* remain viable after exposure to Nebraska weather during a whole winter. The foregoing data indicate that the eggs of Ascaris are resistant to low temperatures as is also shown by Cram's (1924) experiments on refrigeration.

That Ascaris eggs will likewise withstand the conditions found in nature during the summer months is questionable. Ross (1916) found that the eggs of *A. lumbricoides* placed on glass slides and exposed to the sun of India for six weeks contained actively motile embryos at the end of that time. Ransom and Foster (1920), however, reported that

* Contribution from the Department of Helminthology of the School of Hygiene and Public Health, Johns Hopkins University. It is a pleasure to acknowledge the helpful suggestions of Dr. W. W. Cort during the course of these experiments.

the eggs of *A. suum* exposed to 98°F. until extremely dry failed to develop when moistened and removed to a lower temperature. Likewise it was found by Brown (1927) that the eggs of *A. lumbricoides* normally deposited in stools upon sandy soil in direct sunlight during the months of July and August in Panama were all killed before becoming infective. As the above summary would suggest there are considerable data on the longevity of Ascaris eggs. However, due to the absence of an adequate method for isolating eggs from soil, the exposure of the eggs to outdoor conditions necessarily was artificial and not like that actually found in nature where the eggs are intermingled with the soil and are exposed to all its variations in heat and moisture. Although the experiments cited above indicate the high resistance of Ascaris eggs further work more closely approximating natural conditions found in the field in which the temperatures and rainfall are recorded will add considerably to the present knowledge on the question. The purpose of the experiments recorded in this paper was to ascertain the longevity of the eggs of *A. lumbricoides* and *A. suum* when exposed to the outdoor conditions of the winter and spring at Baltimore, Maryland. It was possible to do only a limited series of experiments using only a single type of soil, namely sand, as the seat of the cultures. Many more experiments using different types of soil are needed to complete the study of the longevity of the eggs under all sorts of soil conditions.

## METHODS EMPLOYED

As it was desired to ascertain the longevity of Ascaris eggs under the type of conditions to which they are exposed in nature it was advisable to reproduce these conditions as nearly as possible in the experiments. With this in view a number of small boxes six inches square and six inches deep were made. These were filled with five inches of ordinary sand after small holes had been bored in their bottoms to allow adequate drainage. These boxes were then placed on the roof of the School of Hygiene Building in an angle formed by two walls in such a position as to be exposed to the direct rays of the sun part of the morning and all of the afternoon. Feces with Ascaris eggs were placed on these sand cultures and left completely exposed. As it was desired to learn if the different embryonic stages of development would withstand the conditions of this experiment, eggs in the one cell, morula stage, and with completely developed embryos were used. The eggs were incubated to these later stages in the laboratory. The fact that these experiments were begun in the early winter at a time when the temperature was too low to permit development of the eggs eliminates the possibility of further development once they were placed out of doors, until the warm spring weather. The maximum and minimum temperatures

were taken daily at the culture site. Precipitation data over the period of the experiments were obtained from the local station of the U. S. Weather Bureau. These data are shown in graph I. At monthly intervals a small sample of soil was taken from each culture and the eggs isolated from it by the method of the Caldwells (MSS.). In order to be sure that the undeveloped and partially developed eggs isolated were actually viable they were incubated at 30°C. for 15 days and then examined for active embryos.

The histories of the eggs used in these experiments are as follows:

Culture I.—*A. lumbricoides* from uterus of adult worms collected Aug. 8, 1926, and stored in 7.5% formalin until two weeks before they were used, at which time they were incubated at 30°C. until all were embryonated. Placed on sand culture Dec. 26, 1926.

Culture II.—*A. lumbricoides* from feces of child. Collected Aug. 16, 1926, sedimented; incubated in 2% formalin until embryonated. Placed in sand culture Dec. 9, 1926.

Culture III.—*A. lumbricoides* from feces of child. Collected Aug. 18, 1926, sedimented and stored in 2% formalin until used. Placed on sand culture Nov. 30, 1926, in morula developmental stage.

Culture IV.—*A. suum* from uterus of adult worms. Collected Nov. 29, 1926. Placed on sand culture Nov. 30, 1926.

Culture V.—*A. suum,* same source as IV, incubated in 2% formalin at room temperature until Dec. 9, 1926, until all were in morula stage of development. Placed on sand culture Dec. 9, 1926.

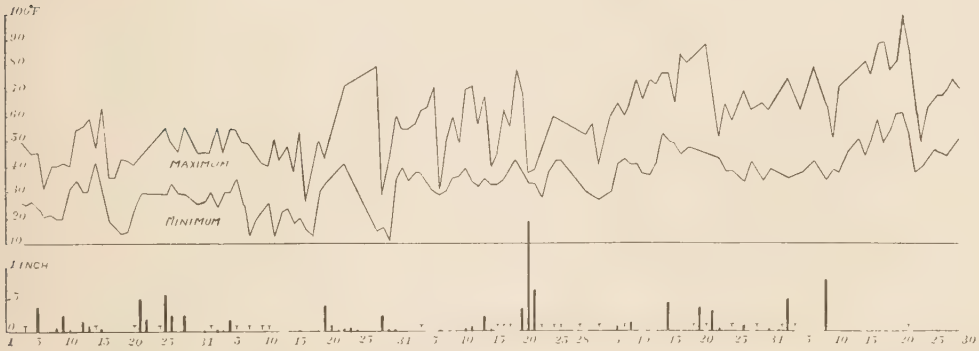
Culture VI.—*A. suum,* same source as IV, incubated in 2% formalin at room temperature until Dec. 26, 1926, when all were embryonated. Placed on sand culture Dec. 26, 1926. The eggs which were obtained from the uteri of worms were mixed with feces before planting on the sand in order to duplicate natural conditions as closely as possible.

## RESULTS OBTAINED

Table 1 summarizes the results of the experiments. Since the cultures were set out on the roof on three different dates they will be considered in groups depending on the date of exposure. Two cultures (III and IV) were begun November 30, 1926, culture III containing the morula stage of *A. lumbricoides* and culture IV *A. suum* in the one cell stage. Isolations from these two cultures at four monthly intervals indicated that the eggs were viable, for when the eggs from an isolation were incubated at 30°C. they contained motile larvae in 10 to 15 days. During these four months the eggs were exposed almost daily to alternate freezing and thawing temperatures (graph I), the range being from 10° to 90°F. During the fifth month of the experiment (April) the temperature was uniformly above freezing and there were several very hot days, on one of which the temperature rose to 101°F. in the sun. Concomitant with this warm weather was a marked decrease in rain. In fact during the whole of this month rain fell only six times in measurable quantity (graph I). This rainfall was all in the first 9 days of the month and during the last 21 days of the month

which was the period of comparatively warm weather, there was no measurable rain whatever. The isolation made at the end of this month from these two cultures showed all the eggs to be dead with their protoplasm markedly degenerate (table 1).

Of the two cultures set out December 9, 1926, one contained the embryonated eggs of *A. lumbricoides* (culture II) and the other eggs of *A. suum* in the morula stages (culture V). These cultures (table 1) were exposed to 10 days less of the cold weather than were cultures III and IV and the isolation on April 5, 1927, showed their eggs were still viable. However, one month later an isolation from them showed that practically all the eggs of these two cultures were dead and degenerating, and on isolation a month later (June 5, 1927) failed to show a single viable egg.




EXPLANATION OF GRAPH

Temperature data obtained at site of cultures during course of experiments (above). From December 1, 1926, through April 30, 1927. Rainfall (in inches) during the time of the experiment. Data from Government Weather Bureau (below). "T" indicates rain fell but not in sufficient quantity to be measured.

Cultures I and VI contained embryonated eggs of *A. lumbricoides* and *A. suum* respectively. These two cultures were placed out of doors on December 26, 1926. The eggs in both cultures withstood the alternate freezing and thawing temperatures of the next three months. However, as with the other cultures, the warm dry weather of April was lethal to them for an isolation made from culture I on May 2, 1927, showed nearly all the embryos within the eggs to be dead and degenerating while all the eggs in culture VI were all dead and degenerating. A check isolation made June 5, 1927, from every culture of the experiment failed to reveal a single viable egg.

## THE EFFECTS OF DRYING ON ASCARIS EGGS

It is of interest that in all the cultures the eggs in all stages remained viable throughout a considerable period of cold weather, yet with warm

weather and alternate wetting and drying they all lost their viability rather quickly. The results in the warm dry weather suggested the need of further experiments, since, as noted in the introduction, the effect of drying upon the viability of Ascaris eggs is not entirely settled.

In order to ascertain more definitely the effect of drying upon Ascaris eggs and to find, if possible, the mechanism of protection or destruction the following experiment was done: Eggs from the uteri of *A. suum* were incubated in a very dilute potassium dichromate solution at 30°C. for 15 days. At the end of this period 100% of the eggs contained actively moving embryos. Small amounts of the fluid containing the eggs were allowed to trickle down the sides of test tubes in such a way that most of the eggs would cling to the sides of the tubes. Likewise eggs were placed on filter paper in petri dishes. The test tubes and petri

TABLE 1.—*Condition of Ascaris Eggs at Monthly Intervals from Cultures in Sand on Roof*

| Culture No. | Egg Species | Stage of Egg Development When Planted | Date Put Out on Soil, 1926 | Isolation Results—Condition of Eggs | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Dec. 30, 1926 | Jan. 31, 1927 | Mar. 1, 1927 | Apr. 5, 1927 | May 2, 1927 | June 5, 1927 |
| I | A. lumbricoides | Embryonated | Dec. 26 | No isolation | Viable | Viable | Viable | Nearly all dead | All dead |
| II | A. lumbricoides | Embryonated | Dec. 9 | No isolation | Viable | Viable | Viable | Nearly all dead | All dead |
| III | A. lumbricoides | Morula | Nov. 30 | Viable | Viable | Viable | Viable | All dead | All dead |
| IV | A. suum | One cell | Nov. 30 | Viable | Viable | Viable | Viable | All dead | All dead |
| V | A. suum | Morula | Dec. 9 | No isolation | Viable | Viable | Viable | All dead | All dead |
| VI | A. suum | Embryonated | Dec. 26 | No isolation | Viable | Viable | Viable | All dead | All dead |

dishes were then separated into two lots and placed on a table near a window, one of the sets being covered with heavy manila paper to protect it from the sun. Control cultures covered with a layer of sodium dichromate solution were placed along with them. A thermometer was placed among the test tubes and occasional temperature records taken.

The moisture from the experimental tubes and petri dishes quickly evaporated leaving the eggs tightly adhering to their containers. At intervals of 9, 28, and 37 days from the beginning of the experiment the eggs were examined. This was done by taking a test tube and petri dish from both "sun" and "shade" experiments, washing the eggs free and examining. It was thought when the experiment was planned that there might be a difference in the effect of drying on Ascaris eggs depending on their exposure to direct sunlight or shade. It was seen, however, after 9 days' drying, that no difference in effect could be detected so the results of the "sun" and "shade" cultures are recorded together.

On January 1, 1927, eggs containing motile embryos were placed in the test tubes and petri dishes and allowed to dry. Eight days later (January 9) examination of test tubes and petri dishes from both the shaded experiment and those exposed to direct sunlight showed that many of the eggs were plainly degenerate. A number of the eggs contained a large bubble, apparently gaseous in nature, which the active embryo forced about as it moved. The controls showed 100% of the eggs with motile embryos. Twenty-eight days from the beginning of the experiment examination of tubes and petri dishes from both the sun and shaded lots showed that practically 100% of the eggs were degenerate. The quiescent larvae appeared vacuolated and transparent. An occasional egg showed a larvae within, sluggishly moving about and forcing the bubble enclosed in the egg from one end of the egg to the other. On February 6, 1927, 37 days from the beginning of the experiment, all the larvae were dead and degenerating, while the controls covered with the dichromate solution contained 100% motile embryos. During the course of the experiments the room temperature was about 23°C. Occasionally during the heat of the afternoon (January) the thermometer in the direct sunlight registered as high as 35°C., but only on several occasions and then for an hour or two.

This experiment indicates that drying of embryonated Ascaris eggs even at rather moderate temperature is lethal to 100% of them after 37 days while some are dead within 9 days. It was noted that the eggs containing the large refractive bubble, presumably gas, when moistened were *all* ruptured rather quickly. It appeared as though the gas bubble was of major importance in this rupture, for eggs which had been dried equally long but which were without this bubble only occasionally ruptured when moistened. This phenomenon of eggs rupturing might in part explain the death of the eggs in the cultures on the roof which were subjected to alternate wetting and drying.

It is interesting to speculate upon the origin of the bubble within the dried eggs. The following method of the origin of this "gas" bubble is presented not as a fact, but as a hypothesis. It is well known that the eggs of *A. suum* will not develop when dry, presumably due to the fact that oxygen is necessary and to secure oxygen for the egg protoplasm the membrane surrounding it must be moist to allow gaseous exchange. The eggs used in this experiment were incubated in water saturated with oxygen so that when the eggs containing the motile embryos were allowed to dry their protoplasm was oxygen saturated as well as the fluid surrounding them inside the shell. Due to the drying of the vitelline membrane of the eggs no further gaseous exchanges between the inside of the egg and the atmosphere could take place. As

a result, as the supply of oxygen in the fluid inside the eggshell was used up by the embryo in the metabolic processes of oxidation, the resulting gaseous products formed may not be as soluble in the fluid bathing the embryo as oxygen; and hence, instead of going into solution in toto, may in part remain undissolved and appear as the large bubble within the egg membrane. The disappearance of large granules within the embryos indicate metabolism during the period of dryness. In corroboration of this theory it was noted that in some cases the longer the eggs were dried the larger the bubble within them grew until it completely filled the interior of the shell.

## DISCUSSION AND SUMMARY

The results of these experiments indicate that Ascaris eggs in all stages of development from both human and pig sources can withstand alternate freezing and thawing as it occurs in nature, over a considerable period of time, at least four months. This fact is of considerable interest especially since Cram (1924) already has shown they will withstand successfully temperatures as low as –17°F. as long as 40 days. Martin's (1926) work showed that eggs in the one cell stage remain viable as long as four years when kept moist at a temperature from –5°C. to 10°C. (23° to 50°F.). It is well known that alternating freezing and thawing are much more fatal to protoplasm than is continuous freezing and the fact that Ascaris eggs can resist them indicates the high resistance of these eggs to low temperatures. It was not definitely proved that the eggs were infective after the exposure to the alternating temperatures. This seems very probable, however, since the embryos all appeared active and normal in every respect. Further, the eggs exposed to these conditions in the early stages, developed normally to the motile embryo stage when given optimum moisture and temperature conditions.

The death of practically all the eggs during April when the weather was rather warm and dry leads to the conclusion that the high temperature along with the extreme dryness were the lethal factors. It may be argued that in nature soil does not drain and consequently dry out as rapidly as did the five inch layer of sand used in the experiments. While this may be partially true the data obtained from experiments under normal soil conditions in Panama (Brown 1927) showed that *sandy* soils in nature, exposed to the direct sunlight lose their moisture very rapidly. It will be seen, however, that the type of experiment recorded here should give a better index to the longevity of Ascaris eggs under field conditions than those in which the eggs are kept in a formalin culture for years. Since only one type of soil, namely sand, was used the conclusions are only valid for this type of soil. Had loam, clay, or

humus soil been used entirely different results might have been obtained as is indicated by the Panama cultures on different soils (Brown 1927). The experiments carried on in the laboratory on the effect of drying upon completely embryonated eggs indicate further that continued drying even when not accompanied by high temperature is lethal.

These experiments check in general those of other workers on the longevity of Ascaris eggs when exposed to outdoor conditions. That the eggs were killed later in the experiment during the dry, warm weather indicates that this is probably what happens in nature under similar conditions and consequently the fatality among Ascaris eggs in nature may be rather high. That other workers did not note dying off of eggs among their experiments in warm weather can probably be attributed to their lack of a method for isolating eggs from soil.

## CONCLUSIONS

1. Eggs of *Ascaris lumbricoides* and *A. suum* in sandy soil cultures out of doors remained viable during the winter months of 1926-1927 at Baltimore, Maryland. During this time the temperature alternated almost daily between freezing and thawing.

2. All stages of embryonic development appeared to withstand the effects of freezing and thawing temperatures equally well.

3. The warm and dry weather of April proved lethal to all of the eggs in all cultures and indicates that in nature, in sandy soil at least, the longevity of Ascaris eggs may be considerably shortened by these conditions.

4. In an experiment in which the embryonated eggs of *A. suum* were dried at room temperature and were exposed to a temperature as high as 35°C. for a short time the embryos were all killed within 37 days. Some were killed at 9 days and at 28 days the great majority of the embryos were dead.

5. Eggs dried for a period contain a bubble of gas which aids in the egg's destruction when moistened. A theory for the formation of this bubble is advanced.

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# INFECTION EXPERIMENTS WITH *HYDRAMOEBA HYDROXENA* NOV. GEN.

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## INTRODUCTION

In the autumn of 1910, Dr. Geza Entz collected some fresh-water polyps, *Hydra oligactis* Pallas, from the pools near Budapest, and brought them into his laboratory. For a while the polyps thrived, but during the latter part of September he noticed that they began to degenerate. Upon making a microscopical examination to determine the cause of this phenomenon he discovered amoebae on the body-wall, peristome, and tentacles and also in the enteric cavity of the hydras. He made a careful study of the amoebae which he published (1912).

Several years ago Dr. F. M. Root observed amoebae on *Hydra viridis* from pools in the vicinity of Baltimore. Hegner and Taliferro (1924 : 84) show a drawing of an amoeba made from his slides. To our knowledge this is the only case recorded from this country, although Professor Geo. R. La Rue verbally informed the senior author of having found hydras, collected from pools near Ann Arbor, Michigan, heavily infected with amoebae during the autumn of 1926.

In the autumn of 1925 the junior author found amoebae on *Hydra oligactis* collected from a pool near the University of Virginia. These were identified as *Amoeba hydroxena* Entz. Although hydras have been studied regularly in the laboratory here for the past twenty-five years, this is the first time the presence of amoebae has been noted. Since then they have been quite abundant, especially during the last quarter of the year; so much so as to constitute a serious threat to the hydra population in some pools. It has been a question of doubt as to whether or not the amoebae are pathogenic to their host. Although Entz observed that they fed on the ectodermal and endodermal cells of their host, he considered this to be more in the nature of "food-robbers," since they also fed on Balantidia which live in the enteron of hydra. Furthermore, he attempted to transfer the infection to other hydras, but usually the experiments were unsuccessful. If however, the transfers were successful, it was of no great significance, for apparently the amoebae attached themselves with difficulty. When transfers were made to hydras from other localities the amoebae did not become attached. In an effort to throw more light on this question we outlined a series of infection experiments.

The experimental animals were cultured in clear spring water (free of inorganic salts) in clean glass vessels. Most of the observations were made on *Hydra oligactis,* six on *H. viridis,* several on *Microstoma caudatum, Stenostoma leucops,* and some on small nemertean worms, species not determined. Transfers were made with clean pipettes. Observations were made under the microscope. When the hydras were fed the nourishment consisted of fresh-water crustacea, segments of aquatic insect larvae, muscle-tissue from grasshopper legs and from the tail of tadpoles. Apparently the most preferred food in this list consisted of fresh-water crustacea, especially Cyclops. The prepared material was fixed in hot Bouin's fluid, imbedded in paraffin, cut into sections 7$\mu$ thick and stained in Heidenhain's iron-hematoxylin.

That the amoebae can readily be transferred from one hydra to another was shown by 65 positive cases out of 66 attempts. Ordinarily the amoebae were placed in the medium in close proximity to the hydra which was to be infected. Twenty-four hours later the hydra was examined and the number of amoebae attached and their position of attachment noted. On twenty animals thus observed there was a total of 29 amoebae on the tips of the tentacles, 63 along the sides of the tentacles, 10 on the peristome and 12 on the body wall. Thus, of the 114 amoebae which had been attached less than twenty-four hours, 92 were on the tentacles and only 22 on the body. This probably can be partially explained as due to the movement of the tentacles, sweeping over the surface to which the animals are attached. The amoebae, having a greater specific gravity than water, sink to the bottom. Moreover, their external surface seems to be slightly covered with a mucilaginous substance, so that they tend to adhere to objects with which they come in contact. The tentacles of hydra are also somewhat sticky. Often amoebae have been seen to adhere lightly to a tentacle as it brushed over them. During the early stages of infection the amoebae are practically always more plentiful on the tentacles than on the body (Fig. 1), but as the infection progresses the parasites become rather generally scattered over the body-surface (Fig. 2).

With proper treatment uninfected hydras will live indefinitely and even without being fed, they will live for three weeks or longer, for as Kepner and Jester (1927) have shown, during periods of inanition they bite off their own tentacles and digest them, later growing a new and smaller set. On the other hand, the hydras which were infected with amoebae died and disintegrated within a relatively short time. For instance, of 48 hydras experimentally infected with four amoebae each:

2 failed to die of the infection, amoebae disappeared
9 died on the third day
9 died on the fourth day
5 died on the fifth day
5 died on the sixth day

6 died on the seventh day
3 died on the eighth day
1 died on the ninth day
3 died on the tenth day
2 died on the eleventh day
1 died on the sixteenth day
1 died on the twenty-first day
1 died on the thirtieth day

The two which lost their infection were reinfected and subsequently succumbed, one on the sixteenth and one on the thirtieth day. In these two cases, especially, there was some indication of a greater resistance to the parasites on the part of the host. From the above table it will be observed that none of the hydras died in less than three days; whereas one lived for thirty days. The average time required to cause death being 6.8 days.

In another series of experiments involving 17 hydras experimentally infected with one amoebae each, death of the host occurred on the average in 12.3 days. In all of these experiments only one hydra died of causes considered to be due to factors other than the presence of amoebae. The amoebae usually divided once or twice during twenty-four hours, thus increasing in numbers very rapidly. If the infection extended over a long period of time the rate of reproduction was not so rapid. The smallest number of amoebae found in a vessel after having caused the death of their host was 20, the greatest 322. The average number in forty-six cases being 126.

In order to ascertain whether or not an abundant food supply is an important factor in resisting amoebic infections, a total of sixteen hydras were exposed to four amoebae each, according to the method described above, and fed daily as long as they were able to eat. Amoebae became attached in every case except one, although two of the others lost their infection during the third day. (In each of these cases only two amoebae became attached). Of the remaining thirteen,

1 died on the third day
3 died on the fourth day
1 died on the fifth day
2 died on the seventh day
2 died on the eighth day
3 died on the tenth day
1 died on the thirtieth day
The average time being 8.5– days

For comparison with these results, we present the records of thirty-three hydra which were infected but were not fed during the period of infection. All of these became infected at the first attempt. Of these

8 died on the third day
6 died on the fourth day
4 died on the fifth day
5 died on the sixth day

4 died on the seventh day
1 died on the eighth day
1 died on the ninth day
2 died on the eleventh day
1 died on the sixteenth day
1 died on the twenty-first day.
The average being 6.1 days.

Thus the hydras which were fed survived the infection for an average of 2.4 days longer than those which were not fed. In addition to this, three of the sixteen hydras which were fed either failed to become infected, or else recovered from a mild infection. Several probable explanations may be offered for this. As the most plausible, we would suggest that the amoebae involved were less capable of producing an infection, or else they were ingested with the food and ultimately killed by the enteric juices. Subsequent infections proved to be fatal to each of these hydras.

In the majority of the experiments the hydras were kept in depression slides, the concavities of which contained 0.25 cc. of water. It occurred to us that the severity of the infections might be due, in part at least, to the relatively small volume of water in which the polyps were living. Accordingly, therefore, we ran other infection experiments in which the hydras were kept in 3 cc. of water, and in still others the polyps were placed in 200 cc. The course of the infections under these three conditions may be briefly summarized as follows:

| | | | | |
|---|---|---|---|---|
| 31 hydras in | 0.25 cc. | of water died on average in | 8 | days |
| 5 hydras in | 3.00 cc. | of water died on average in | 6.4 | days |
| 10 hydras in | 200.00 cc. | of water died on average in | 3.5 | days |

From the above data it appears that the infection is more severe in a large quantity of water than in smaller volumes. This may be due to the fact that the host is more distended when its confines are not narrowly limited, thus affording a better opportunity to the amoebae for extracting cells upon which they feed. There is this advantage, however, when the polyps are in large bodies of water: viz., the amoebae have less chance of encountering them. This was shown experimentally when five hydra were placed in 200 cc. of water containing 20 amoebae. In this case only three of the hydras became infected. From our experience in observing these forms we are of the opinion that, in a large pool of water, a hydra would not be infected very readily so long as it remained near the surface or attached to a blade of grass or other object above the bottom.

Entz states that *A. hydroxena* crawls around in the enteron as well as on the body surface. It is not an uncommon thing to find amoebae in the enteric cavity of sectioned infected hydras. Entz does not say how the amoebae get inside, but we have seen them carried in from the

peristomal region on particles of food. It is also probable that they may be taken in on tentacles which, as has been stated above, are occasionally bitten off.

In an effort to ascertain whether or not the amoebae can remain alive and produce an infection in the enteric cavity, we injected from 2 to 10 amoebae through the mouth into the enteron of each of sixteen hydras. The injections were made by means of a capillary pipette (caliber 200$\mu$) under a binocular microscope. The hydras were then carefully examined—any amoebae on the external surface or in the medium being removed. Next they were washed in spring water and then transferred to a dish of clean spring water, where they were further examined both with the binocular and compound microscopes. One hour after the injection, one of the hydras was fixed, and then sectioned and stained. In this animal four amoebae were found near the middle of the enteron. Five of the hydras were fed one hour after being injected and daily thereafter for four days. On the third day one of these had disgorged its enteric contents and fifty amoebae were found on its external surface. Two days later the hydra disintegrated and 218 amoebae were found in the containing vessel. The other four remained healthy during the rest of the experiment i.e. for 15 days, three of them producing each an offspring vegetatively and two producing each a normal egg.

The remaining ten injected hydras were not fed. Twenty hours after being injected one of these was fixed and then sectioned and stained. In this animal one large amoeba was found. Two days after being injected another one of these was fixed, sectioned and stained, but no amoebae were found in it. Three days afer being injected another specimen was fixed and sectioned, and it too failed to have amoebae present. Another specimen similarly examined on the sixth day after being injected gave negative results also, as did specimens examined on the tenth and eleventh days respectively.

Of the fifteen injected specimens, kept for one day or longer, eight showed amoebae on the external surface for from one to seven days after the injection. Evidently these came from the inside. They may have escaped either by forcing their way between the peristomal lips, or else by being ejected along with other enteric contents by their hosts. Each day the hydras were carefully examined and if amoebae were present they were removed by means of a small pipette. These experiments indicate that although amoebae can live and evidently multiply in the enteron of hydra for one, and probably seven days, they are, nevertheless, incapable of serving as truly entozoic parasites.

If the amoebae ingest other protozoa as claimed by Entz, it seems reasonable to believe that they may lead a free-living existence. This, however, apparently is not the case. In our observations involving thousands of amoebae we have never seen them ingest unicellular ani-

mals. Whereas they are able to live for several days, entirely removed from hydras, it has been our experience that they disappear from the medium in from four to ten days after the separation. Also, we have found them to be non-infective after the sixth or seventh day. When first taken from their host they are full of food, somewhat stubby, and usually possess many short digitate pseudopods. Two or three days later they assume a slug-like form and resemble very much the limax-type of amoeba. After being removed from their host for five or six days many of them exhibit a spherical vacuolated appearance, and shortly thereafter they disappear, apparently giving rise to many amoebulae of about 10$\mu$ diameter.

Attempts to transfer the amoebae to different host species were rather limited in number. In six cases *Hydra viridis* was infected with amoebae from *H. oligacitis*. The infection resulted in death in every instance, but the few experiments performed indicated that this species of polyp is slightly more resistant to the amoebae than is *H. oligactis*. After feeding on the endodermal tissue the amoebae were green, due to the presence of zoochlorellae from their hosts cells in food vacuoles. By the following day these plant cells would be eliminated from the amoebae's bodies, apparently undigested.

Out of forty attempts to transfer the amoebae to *Microstoma caudatum*, by placing ten amoebae with each microstoma in a depression slide, only ten amoebae became attached. The attachments were always made along the lateral margin about one third the distance from the posterior end. All of these attachments were temporary, never lasting more than two days. Efforts were also made to infect Microstoma internally by feeding them tentacles of hydra containing amoebae. In one case the Microstoma was fixed and then sectioned two hours after having eaten the amoebae. What was taken to be the remains of one amoeba was found. In another instance the Microstoma was fixed eighteen hours after having ingested the amoebae. No evidence of amoebae was found in the sectioned animal. In another experiment the Microstoma was observed for two weeks after it was fed amoebae on hydra's tentacles. During this time it gave rise to two generations of zooids, but no evidence of amoebae was ever apparent.

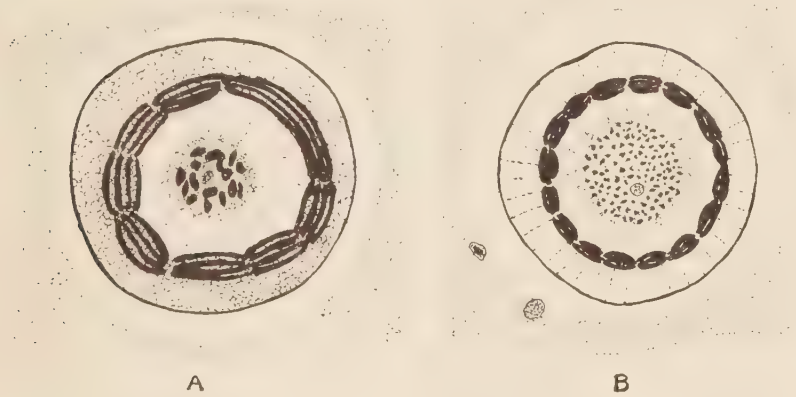
Eighteen attempts were made to infect *Stenostoma leucops* with amoebae from hydra in which over two thousand amoebae were used. Only six of these became attached. The attachments were made along the lateral margin and were very transitory. Efforts to transfer the amoebae to small nemertian worms proved to be entirely unsuccessful.

## DISCUSSION

By the above experiments it has been shown that the amoebae are highly pathogenic to hydras. In the article describing this species Entz

concludes that it is not a truly disease-producing form. For that reason, and because it possesses certain features in common with the free-living amoebae, notably a contractile vacuole, he places it with the free-living species in the genus *Amoeba*. Entz's general description fits the form dealt with in this investigation so well that we are doubtful about their being different species. There are a few differences, however, which might be enumerated.

| | *Entz's amoebae* | *This amoeba* |
|---|---|---|
| Length: | 100-380μ | 60-190μ |
| Nuclear size: | 14-30μ | 10-18μ |
| Food: | Hydra cells and ciliates | Hydra cells |
| Effect on host: | Not pathogenic (?) | Very pathogenic |




Text Fig.—Somewhat generalized drawings of the nuclei in optical section of Entz's amoeba and the one involved in this study. The differences shown may be due to differences in fixation, interpretation, or real morphological differences. Note perinuclear vacuole, nuclear membrane, karyosome with two kinds of chromatin, and centrosome (?). *A*. Copied from Entz's drawing. *B*. Drawing based on conditions found in our slides.

Although some of the differences noted above are rather pronounced, we are not inclined to consider them as being sufficiently great to justify the establishment of a new species. Aside from Entz's general description being so applicable to the form we worked with, the two are alike further in that their seasonal period is strikingly similar. For instance, Entz speaks of his amoeba as appearing in numbers during the latter part of September and disappearing between the sixth and eighth of December. This is approximately the picture of the situation here under natural conditions. In our cultures, however, we can see no special seasonal variations in regard to the viability of the amoebae.

Since this form is distinctly not a free-living species and since it is not related to the other parasitic genera, neither morphologically nor physiologically, we propose to erect a new genus to contain it and sug-

gest as the generic name Hydramoeba, retaining the specific name *hydroxena*. The proper name of this species is, therefore, *Hydramoeba hydroxena*.

SUMMARY

1. *Amoeba hydroxena* is placed in a new genus, Hydramoeba, due to the fact that it is a pathogenic parasite and differs morphologically and physiologically from existing parasitic genera of amoebae.

2. Out of 66 attempts to infect hydras experimentally with *Hydramoeba hydroxena*, death of the host was caused in 65 cases, the length of time required ranging from three to thirty days.

3. The amoebae are not able to live indefinitely removed from hydras but disappear from the medium in from four to ten days.

4. The amoebae are pathogenic to both *Hydra oligactis* and *H. viridis*, but attempts to infect Stenostoma, Microstoma, and small nemertean worms were unsuccessful, though a few amoebae became temporarily attached to the rhabdocoeles.

5. The general characteristics of *Hydramoeba hydroxena* are as follows: An amoeba ranging in size from 60 to 190$\mu$ with an average of 123$\mu$ (Entz's forms ranged from 100 to 380$\mu$), with a vesicular type of nucleus, possessing one or more contractile vacuoles, living primarily as an ectozoic parasite on Hydra, feeding on the cells of its host, and causing death on the average in 6.8 days.

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EXPLANATION OF PLATE III

Fig. 1.—Living *Hydra oligactis* during early stage of infection, four amoebae on tentacles and one on body wall. Note that one amoeba is attached to two tentacles. These tentacles were held together by this amoeba for over an hour. They were then forcibly separated with the aid of needles, but the attachment of the amoeba was so secure that it was torn into two parts. × 70.

Fig. 2.—Camera lucida sketch of stained hydra during late stage of infection, showing loss of tentacles and rounded condition of body with numerous amoebae on surface. At this stage there are probably many amoebae in the enteric cavity also. × 140.

Fig. 3.—Sectioned specimen of *Hydramoeba hydroxena* on external surface of *Hydra oligactis*, showing extensive erosion of ectodermal cells. In the amoeba may be seen a nematocyst, while two cell nuclei are partly surrounded by pseudopods. × 700.

Fig. 4.—Sectioned specimen of *H. hydroxena* in the enteron. The amoeba's cytoplasm is filled with numerous food bodies. The distal ends of several endodermal cells are apparently being ingested and two cell nuclei may be seen near the encroaching pseudopods. × 700.

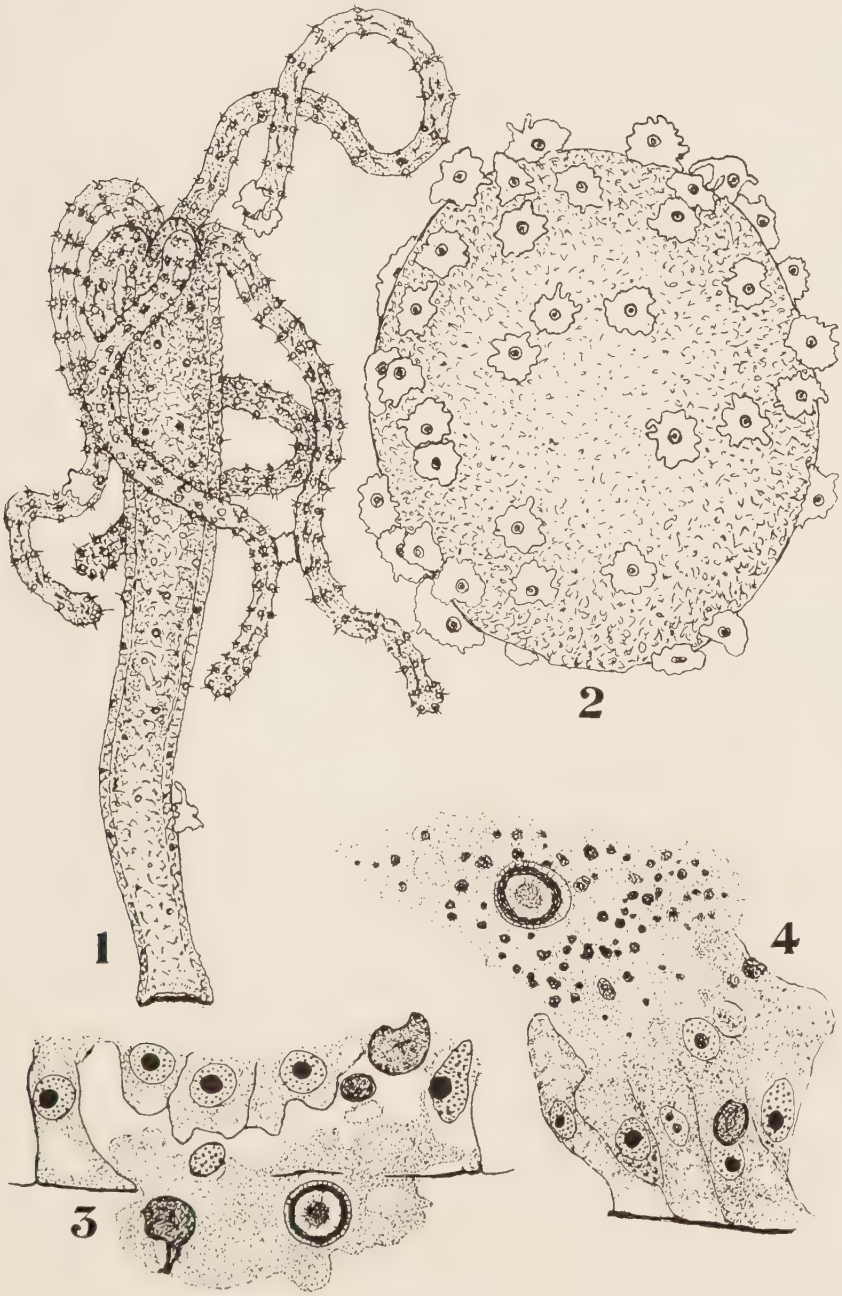


PLATE III

# SOME INTESTINAL AMOEBAE AND FLAGELLATES FROM THE CHIMPANZEE, THREE-TOED SLOTH, SHEEP AND GUINEA-PIG*

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## ENDAMOEBAE FROM THE CHIMPANZEE

An examination of a captive, but non-dysenteric, chimpanzee that died in the zoological garden in Druid Hill Park, Baltimore, revealed the presence of three types of amoebae, (1) cysts resembling those of *E. histolytica,* (2) cysts like those of *E. coli,* and (3) cysts and trophozoites of a species similar to *Iodamoeba williamsi.* Histolytica-like and coli-like amoebae have been reported from the chimpanzee, but, so far as known to the writers, this is the first record of an Iodamoeba from this type of monkey. Endamoebae resembling *E. histolytica* have been reported from the gorilla (Wenyon, 1926), chimpanzee (Suldey, 1924; Deschiens, 1927) and various other species of Old World and New World monkeys. Coli-like amoeba were described by Deschiens (1927) in five of seven chimpanzees at the Pasteur Institute in Paris; they have also been noted by Brumpt (1909) in *Macacus sinicus,* by Prowazek (1912) in the orang, and later by other investigators in a number of other species of Old World and New World monkeys. Altogether, at least eleven species of amoebae belonging to three genera have been reported from monkeys. Nine of these have been placed in the genus Endamoeba.

Histolytica-like amoebae. The name *Endamoeba nuttalli* was proposed by Castellani (1908) for a histolytica-like species that he found in a liver abscess in a *Macacus pileatus* monkey in Colombo, Ceylon. Data presented in the literature since then are insufficient to separate this so-called species from *E. histolytica* from man; and the other amoebae with histolytica-like trophozoites and four-nucleated cysts that have been described from monkeys as *E. duboscqi* (Mathis, 1913), *E. chattoni* (Swellengrebel, 1914), *E. ateles* (Eichhorn and Gallagher, 1916) and *E. cynomolgi* (Brug, 1923) may really be *E. nuttalli,* which likewise may be a synonym of *E. histolytica.*

Cysts only of the histolytica-like amoeba were found by the writers in the chimpanzee examined. These resembled those of *E. histolytica*

* From the Laboratory of Protozoology. The writers are indebted to Mr. Conrad Bauer for obtaining and preparing material for study.

from man in structure (Fig. 1). Of twenty-five cysts measured, one possessed two nuclei and the other twenty-four, four. The following dimensions and biometrical data were obtained from these measurements:

Diameter, range, 7-16$\mu$; mean, 11.88$\mu$; Standard deviation, 0.84 $\pm$ 0.08$\mu$; Coefficient of variation, 7.07 $\pm$ 0.68%.

Coli-like amoebae. Prowazek (1912) proposed the name *Endamoeba pitheci* for an amoeba with trophozoites similar to those of *E. coli* from man and with eight-nucleated cysts that he obtained from a young orang. Species names have since been given to coli-like amoebae from monkeys as follows: *E. legeri* (Mathis, 1913), *E. cercopitheci* (Macfie, 1915), and *E. multinucleata* (Mello, 1923). These may all belong to the species *E. pitheci,* and the latter may be a synonym of *E. coli.* Twenty-three of the twenty-five cysts from the chimpanzee that were measured contained eight nuclei (Fig. 2), one had four nuclei and the remaining specimen, two. The following dimensions and biometrical data were obtained from these measurements:

Diameter, range, 13-18$\mu$; mean, 15.48$\mu$; Standard deviation, 1.53 $\pm$ 0.14$\mu$; Coefficient of variation, 9.85 $\pm$ 0.95%.

### IODAMOEBA FROM THE CHIMPANZEE

Brug (1921) noted amoebae in *Macacus cynomolgus* that appear to belong to the genus Iodamoeba. Similar amoebae have since been reported from both Old World and New World monkeys (Hegner and Taliaferro, 1924, in *Cebus variegatus;* Kessel, 1924, 1927, in Macacus; Wenyon, 1926, in the gorilla; and Smith, 1927, in *Macacus rhesus*), but, so far as known to the writers, no Iodamoebae have been described from the chimpanzee. Only a few cysts and trophozoites were found. Measurements of seven cysts (Fig. 3) gave the following dimensions and biometrical data.

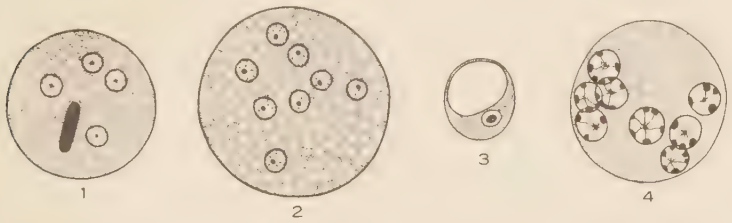
Diameter, range, 7-8.2$\mu$; mean, 7.45 $\pm$ 0.1$\mu$; Standard deviation, 0.41 $\pm$ 0.07$\mu$; Coefficient of variation, 5.48 $\pm$ 0.99%.

The trophozoites were similar to those of *Iodamoeba williamsi* of man. They were irregular in shape and measured about 8 by 7.5 microns in diameter. Brug gave the species named *kueneni* to the specimens he described because of the presence of a darkly staining area not present in cysts from man, but this characteristic may not be constant and, in the writers' opinion, no satisfactory evidence has yet been produced that the Iodamoebae of monkeys and man do not belong to the same species.

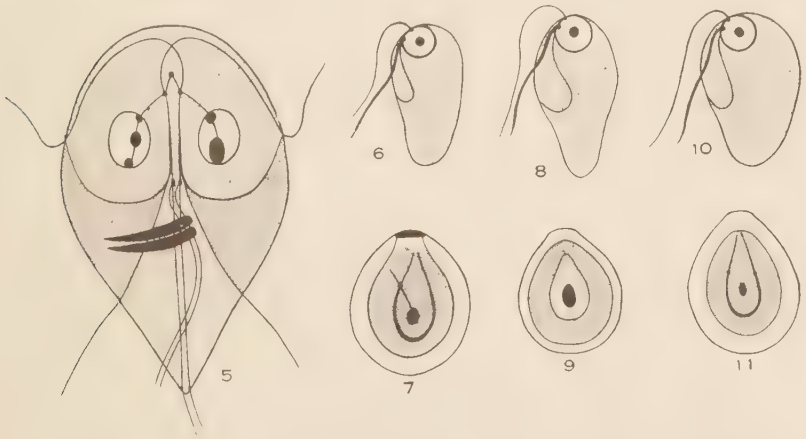
### *Endamoeba bradypi* n.sp. from the Sloth

Several species of intestinal protozoa were found in a specimen of a three-toed sloth *(Bradypus griseus griseus)* that was brought to

Baltimore from Panama by Dr. Curt P. Richter and died about three weeks after its arrival. The cyst of an Endamoeba was observed on slides fixed in Schaudinn's fluid and stained with iron-hematoxlyin (Fig. 4). It measured 14$\mu$ in diameter and possessed eight nuclei 3$\mu$ in diameter of the histolytica type, but differing from those of *E. histolytica* with respect to the number and size of the chromatin




A.—1. Cyst from the chimpanzee resembling that of *Endamoeba histolytica* from man; × 1,500. 2. Cyst from the chimpanzee resembling that of *Endamoeba coli* from man; × 1,500. 3. Cyst from the chimpanzee resembling that of *Iodamoeba williamsi* from man; × 1,500. 4. Cyst of *Endamoeba bradypi* n.sp. from the sloth; × 1,500.




B.—5. Trophozoite of *Giardia bradypi* n.sp. from the slot; × 4,000. 6. Trophozoite of *Embadomonas bradypi* n.sp. from the sloth; × 4,000. 7. Cyst of *Embadomonas bradypi* n.sp. from the sloth; × 4,000. 8. Trophozoite of *Embadomonas caviae* n.sp. from the guinea-pig; × 4,000. 9. Cyst of *Embadomonas caviae* n.sp. from the guinea-pig; × 4,000. 10. Trophozoite of *Embadomonas ovis* n.sp. from the sheep; × 4,000. 11. Cyst of *Embadomonas* ovis n.sp. from the sheep; × 4,000.

granules on the nuclear membrane. The karyosome of the sloth amoeba is very small, centrally located, and attached to the nuclear membrane by fine achromatic threads. The chromatin granules on the nuclear membrane are few in number (4, 5 or 6), comparatively large, and

spherical or ovoidal in shape. We propose the name *Endamoeba bradypi* n.sp. for this species and designate the three-toed sloth, *Bradypus griseus griseus,* as the type host.

*Giardia bradypi* n.sp. from the Sloth

The same animal in which the amoeba described above was found also contained trophozoites of Giardia (Fig. 5). No giardias have heretofore been described from this animal. This species of Giardia resembles in shape those from the rabbit *(G. duodenalis)* and guinea-pig *(G. caviae),* exhibiting the greatest diameter across the lateral shields. The parabasal bodies are long, curved and blunt at one end and pointed at the other. The following dimensions and biometrical data were obtained from measurements of 100 trophozoites.

| | Length in Microns | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Breadth in Microns | 10 | 11 | 12 | 13 | 14 | 15 | 16 | |
| 6 | | 1 | 3 | | 2 | | | 6 |
| 7 | 1 | 5 | 18 | 21 | 5 | | | 50 |
| 8 | | | 4 | 8 | 18 | 1 | 1 | 32 |
| 9 | | | | 3 | 7 | 2 | | 12 |
| | 1 | 6 | 25 | 32 | 32 | 3 | 1 | 100 |

| | Length | Breadth |
|---|---|---|
| Range | 10-16$\mu$ | 6-9$\mu$ |
| Mean | 13.01 $\pm$ 0.07$\mu$ | 7.5 $\pm$ 0.05$\mu$ |
| Standard deviation | 1.05 $\pm$ 0.05$\mu$ | 0.78 $\pm$ 0.04$\mu$ |
| Coefficient of variation | 8.07 $\pm$ 0.38% | 10.4 $\pm$ 0.5% |

In order that the dimensions of this species may be compared with those previously published for giardias from other species of animals, the following average measurements of ten specimens were obtained. Length of body, 12.51$\mu$; breadth of body, 7.66$\mu$; anterior end to center of nucleus, 3.76$\mu$; center of nucleus to end of lateral shields, 5.4$\mu$; end of lateral shields to posterior end, 3.33$\mu$; breadth across end of lateral shields, 3.93$\mu$; breadth across center of lateral shields, 6.31$\mu$.

We propose the name *Giardia bradypi* n.sp. for this species and designate the three-toed sloth, *Bradypus griseus griseus,* as the type host.

*Embadomonas bradypi* n.sp. from the Sloth

Embadomonads have been described from insect larvae, water bugs, frogs, tortoises and from the following mammals: man (Wenyon and O'Connor, 1917, and others), monkeys (Fonseca, 1917; Kessel, 1927), rabbits (Collier and Boeck, 1926), and guinea-pigs and wild rats (Wenyon, 1926). The sloth in which the Endamoeba and Giardia described above were found also contained embadomonads. These flagellates were found in cultures of serum-saline-citrate medium inoculated with

material from the intestine of the sloth and incubtated at 36°C. Both trophozoites and cysts appeared in the cultures. Nothing particularly distinctive was found in the structure of the trophozoites of this species (Fig. 6), but the cysts differ from any of those thus far described. The darkly staining lines within the cyst (Fig. 7) probably represent threads that support the peristome of the trophozoites, and the nucleus, but could not be distinguished clearly in our preparations. The most con-

Trophozoites of *Embadomonas bradypi* n.sp. from the Sloth

| Breadth in Microns \ Length in Microns | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | |
|---|---|---|---|---|---|---|---|
| 1.6 | 2 | | | | | | 2 |
| 2 | 3 | 4 | 3 | | | | 10 |
| 2.4 | 1 | 2 | 5 | | 1 | 1 | 10 |
| 2.8 | | | | 1 | 1 | | 2 |
| 3.2 | | | 1 | | | | 1 |
| | 6 | 6 | 9 | 1 | 2 | 1 | 25 |

| | Length | Breadth |
|---|---|---|
| Range | 4-6.5μ | 1.6-3.2μ |
| Mean | 4.8 ± 0.09μ | 2.24 ± 0.05μ |
| Standard deviation | 0.66 ± 0.06μ | 0.36 ± 0.03μ |
| Coefficient of variation | 13.75 ± 1.33% | 16.07 ± 1.57% |

Cysts of *Embadomonas bradypi* n.sp. from the Sloth

| Breadth in Microns \ Length in Microns | 4.3 | 4.5 | 4.7 | 4.9 | 5.1 | 5.3 | 5.5 | |
|---|---|---|---|---|---|---|---|---|
| 3.6 | 1 | 1 | 1 | | | 1 | | 4 |
| 3.8 | 1 | 2 | 1 | | | | | 4 |
| 4 | | | 1 | 1 | 1 | | | 3 |
| 4.2 | | 1 | 1 | 4 | 1 | 1 | 2 | 10 |
| 4.4 | | | 1 | | 2 | 1 | | 4 |
| | 2 | 4 | 5 | 5 | 4 | 3 | 2 | 25 |

| | Length | Breadth |
|---|---|---|
| Range | 4.3-5.5μ | 3.6-4.4μ |
| Mean | 4.88 ± 0.04μ | 4.04 ± 0.05μ |
| Standard deviation | 0.28 ± 0.03μ | 0.36 ± 0.03μ |
| Coefficient of variation | 5.73 ± 0.55% | 8.91 ± 0.86% |

spicuous difference between the cysts of this and other species of Embadomonas is the presence of a deeply stained pad at the anterior end that may be a swelling of the cyst wall or part of the cytoplasm that is attached to the cyst wall and from which the body of the flagellate was drawn away during the process of fixation. The following dimensions and biometrical data were obtained from measurements of this species.

We propose the name *Embadomonas bradypi* n.sp. for this species and designate the three-toed sloth, *Bradypus griseus griseus*, as the type host.

*Embadomonas caviae* n.sp. from the Guinea-Pig

Embadomonads were noted by Wenyon (1926) in guinea-pigs. During the four months of November, 1927, to February, 1928, cultures in serum-saline-citrate medium were inoculated with material from the cecum of fifty-two guinea-pigs obtained from four different sources. Fourteen of these were positive for Embadomonas, indicating that these

Trophozoites of *Embadomonas caviae* n.sp. from the Guinea-Pig

| Breadth in Microns | Length in Microns: 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | |
|---|---|---|---|---|---|---|---|---|
| 2 | | | 2 | | | | | 2 |
| 2.4 | 2 | 1 | 1 | | 2 | | 1 | 7 |
| 2.8 | | 1 | 2 | 2 | 2 | 1 | 1 | 9 |
| 3.2 | | 1 | | 1 | 2 | | 1 | 5 |
| 3.6 | | | 1 | | | | | 1 |
| 4 | | | | | 1 | | | 1 |
| | 2 | 3 | 6 | 3 | 7 | 1 | 3 | 25 |

| | Length | Breadth |
|---|---|---|
| Range | 4-7$\mu$ | 2-4$\mu$ |
| Mean | 5.5 $\pm$ 0.12$\mu$ | 2.78 $\pm$ 0.06$\mu$ |
| Standard deviation | 0.86 $\pm$ 0.08$\mu$ | 0.47 $\pm$ 0.04$\mu$ |
| Coefficient of variation | 15.6 $\pm$ 1.52% | 16.9 $\pm$ 1.65% |

Cysts of *Embadomonas caviae* n.sp. from the Guinea-Pig

| Breadth in Microns | Length in Microns: 3.4 | 3.7 | 4.0 | 4.3 | 4.6 | 4.9 | 5.2 | |
|---|---|---|---|---|---|---|---|---|
| 3.0 | | 1 | | | | | | 1 |
| 3.2 | 1 | | 4 | | | | | 5 |
| 3.4 | 1 | 1 | 3 | 3 | | | | 8 |
| 3.6 | | | 2 | 1 | 1 | 1 | 1 | 6 |
| 3.8 | | | 1 | 1 | 1 | | | 3 |
| 4.0 | | | | | | 2 | | 2 |
| | 2 | 2 | 10 | 5 | 2 | 3 | 1 | 25 |

| | Length | Breadth |
|---|---|---|
| Range | 3.4-5.2$\mu$ | 3.0-4.0$\mu$ |
| Mean | 4.19 $\pm$ 0.06$\mu$ | 3.49 $\pm$ 0.03$\mu$ |
| Standard deviation | 0.45 $\pm$ 0.04$\mu$ | 0.22 $\pm$ 0.02$\mu$ |
| Coefficient of variation | 10.7 $\pm$ 1.03% | 6.3 $\pm$ 0.6 % |

flagellates are common and widespread among guinea-pigs. They are probably ordinarily overlooked because of their small size. Both trophozoites (Fig. 8) and cysts (Fig. 9) were obtained in our cultures in sufficient numbers for statistical measurements. Cysts similar in morphology to those of other species of Embadomonas were present. The dimensions and biometrical data secured from trophozoites are as follows.

We propose the name *Embadomonas caviae* n.sp. for this species and designate the guinea-pig as the type host.

*Embadomonas ovis* n.sp. from the Sheep

Both trophozoites (Fig. 10) and cysts (Fig. 11) of Embadomonas appeared in cultures made from the feces of sheep. The dimensions and biometrical data obtained from these are as follows.

Trophozoites of *Embadomonas ovis* n.sp. from the Sheep

| Breadth in Microns | Length in Microns 4.5 | 4.9 | 5.3 | 5.7 | 6.1 | |
|---|---|---|---|---|---|---|
| 2.7 | | 2 | 3 | | | 5 |
| 3.1 | 1 | 2 | | 1 | | 4 |
| 3.5 | 3 | 2 | 1 | 5 | 2 | 13 |
| 3.9 | | | 1 | | | 1 |
| 4.3 | | | | | 1 | 1 |
| 4.7 | | | 1 | | | 1 |
| | 4 | 6 | 6 | 6 | 3 | 25 |

| | Length | Breadth |
|---|---|---|
| Range | 4.5-6.1μ | 2.7-4.7μ |
| Mean | 5.27 ± 0.06μ | 3.37 ± 0.06μ |
| Standard deviation | 0.46 ± 0.04μ | 0.48 ± 0.05μ |
| Coefficient of variation | 8.72 ± 0.83% | 14.2 ± 1.38% |

Cysts of *Embadomonas ovis* n.sp. from the Sheep

| Breadth in Microns | Length in Microns 4 | 4.4 | 4.8 | 5.2 | 5.6 | 6 | |
|---|---|---|---|---|---|---|---|
| 3.1 | | | | 1 | | | 1 |
| 3.4 | 2 | | | | | 1 | 3 |
| 3.7 | 1 | 4 | 2 | 2 | 1 | 2 | 12 |
| 4.1 | | 2 | 2 | 2 | | 1 | 7 |
| 4.4 | | | 1 | | | 1 | 2 |
| | 3 | 6 | 5 | 5 | 1 | 5 | 25 |

| | Length | Breadth |
|---|---|---|
| Range | 4-6μ | 3.1-4.4μ |
| Mean | 4.96 ± 0.09μ | 3.8 ± 0.05μ |
| Standard deviation | 0.65 ± 0.06μ | 0.4 ± 0.04μ |
| Coefficient of variation | 13.1 ± 1.27% | 10.5 ± 1.01% |

We propose the name *Embadomonas ovis* n.sp. for this species and designate the sheep as the type host.

## References to Literature

Complete references to all of the literature on the amoebae of monkeys cited in this paper are included in the following:

Hegner, Robert. 1928.—The evolutionary significance of the protozoan parasites of monkeys and man. Quart. Rev. Biol., 3.

References to literature on Embadomonas are included in the following:

Wenyon, C. M. 1926.—Protozoology. 2 vols. 1563 pp. London.

# ON THE STRUCTURE AND LIFE HISTORY OF AN ADULT *TRIAENOPHORUS ROBUSTUS*

ARTHUR LORIMER HJORTLAND *

Through the kindness of Dr. Ward, I was able to collect material for this manuscript, while working under his personal direction at Ely, Minnesota, during July, August and September, 1927. The cestode, *Triaenophorus robustus* Olsson (1892), was recovered from the *Leucicthys tullibee* and *Esox lucius* in lakes near Ely, Minnesota.

The larvae of *Triaenophorus robustus* were found encysted in the musculature of *Leucicthys tullibee*. These cysts, as described by Cooper (1918), are always located in the musculature of the host from a short distance back of the dorsal fin to close behind the skull. However, the positions of the cysts were different from those recorded by Cooper. The fish which I examined contained such a large number of cysts lying in all directions, assuming various shapes, that from all observations there can be no relation in location between cyst and host.

In the 37 specimens examined, the number of cysts varied from 0 to 70. The following table shows the localities from which the fish were taken and the number of cysts found.

Record of Larvae of *Trienophorus robustus* taken from *Leucicthys tullibee*

| Name of lake | No. of spec. examined | Size of fish | No. of cysts |
|---|---|---|---|
| Long Lake | 15 | 12 to 14 in. | 3 to 35 |
| Fall Lake | 20 | 10 to 14 in. | 7 to 70 |
| Burntside Lake | 1 | 4 in. | 0 |
| Vermillion Lake | 1 | 7 in. | 2 |

The above table shows a relatively high infestation, the average being about 25 cysts.

The cysts were of a whitish opalescent color, varying in size from 3 mm. to 5 cm. in length, and from 2 to 5 mm. in diameter. In general they are cylindrical in shape with blunt ends, but due to crowding, the shapes were very much distorted. A few were found ending in a threadlike structure which was hollow, but apparently this peculiarity was of no consequence.

The size or shape of the cyst does not in any way govern the size of the larvae. In one cyst, 4 mm. in length, the larvae uncoiled measured 5 cm., while in a 2 cm. cyst the larva was less than 1 cm. in length.

* Contribution from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 326.

In all cases the larvae lay in loops or coils surrounded by a yellowish fluid, which was not analyzed. It is my opinion that this fluid is a secretion from the larvae. In preserved specimens this fluid had dried up leaving a caseous residue.

The position of the head in relation to the axis of the cyst was noted. In some specimens the head was found in the extremity of either end, in others in the center of the cyst, while in others the head lay within the innermost coils of the larva. It would seem that the location of the cyst, as well as the arrangement of the worm within, is of little importance in its development.

Cross sections (fig. 1) showed the cyst to be made up of a thick laminated wall about 0.24 mm. thick. The wall is formed by cells forming countless strands of tissue arranged in a circular band. Two types of cells were evident, small round cells and numerous elongated cells. There was no trace of a cyst lining or an outer covering. The cyst was simple in structure and similar throughout.

The cells of the muscular tissue, (fig. 1) surrounding the cyst, did not show any great abnormalities with the exception of a crowding together. The strands of the muscle fibers appear more compact. In none of the cross sections made was there any evidence of giant cells.

The living larva (fig. 1) within the cyst is white in color, presenting an unsegmented body. The body wall is quite strongly and deeply convoluted due to the action of the longitudinal muscles. The depressions or folds begin immediately behind the scolex and continue the entire length of the body.

The length of the entire specimen varies. Of the 755 larvae removed, approximately 200 specimens were measured. The shortest entire specimen found measured 1 cm. in length and 0.925 mm. in width, while the longest one measured 37 cm. in length and 1.5 mm. in width, the greatest width occurring near the center of the body. At the posterior end, an indentation is found showing the location of the excretory pore.

Olsson and Cooper both speak of the larva being provided with a narrow cylindrical "cauda," a tail-like process which is so greatly attenuated that near the posterior end it resembles a thread-like structure. Olsson described one of these as 120 mm. long, the anterior portion approximately 60 mm. long. Cooper gives the measurements of a specimen with a tail 110 mm. long. In all the specimens I examined very few of this type were found. In one instance, a larva measuring 70 mm. long had a "cauda" measuring 100 mm. long. This condition may be characteristic of very young forms. As most of the specimens examined were quite highly developed, it may be that the "cauda" is used up in the development of the cestode.

The scolex as described by Olsson and later by Cooper is in the form of a truncated rectangular pyramid, the terminal disc forms a cap,

underneath which one finds the four tridents of hooks. Extending from the terminal disc to the base of the scolex, on both the dorsal and ventral sides, the bothria can be distinguished, conforming in general to the shape of the entire scolex. They are prominent, yet of no appreciable depth.

In general the scolex is large. The following measurements represent the average scolex found. The anterior end measured 0.925 mm. tapering to a width of 0.825 mm. From here it broadens out until its greatest width, which is at the base, is 1.20 mm. The entire length of the scolex is 1.19 mm.

In the following table a comparison is made of the measurements of scolices, as found by Fuhrmann, Cooper and me. It will be seen that from my collection the specimens had a wider range, some being almost identical with the smaller forms described by Cooper, others more nearly like the large characteristic forms described by Fuhrmann.

*Measurements of Scolex of Larval Form*

| *Triaenophorus robustus* | Hjortland | Fuhrmann | Cooper |
|---|---|---|---|
| Length of scolex | 0.98-1.19 mm. | 1.14-1.50 mm. | 0.98 mm. |
| Width of terminal disc | 0.84-0.92 mm. | 0.95 mm. | 0.77-0.83 mm. |
| Width of scolex posteriorly | 1.20-1.30 mm. | 1.40-1.50 mm. | 1.05-1.30 mm. |

The four tridents of hooks lie just beneath the terminal disc. Each trident is heavy in appearance, quite large and is made up of a chitinous material. The trident is crescent-shaped with a slight indentation on the margin of the basal piece. Three hooks of different sizes are arranged on the inner side of the crescent, two larger ones on either side of a smaller central one. The entire trident extends almost three-fourths of its width into the scolex. The full length of the larger hooks project through the cuticula, while about only one-half of the median one acts as a functional tip. The following measurements are of a typical trident: width of trident, 0.27 mm., its medial length, 0.16 mm., while the part which lies toward the external part of the scolex is 0.18 mm. in length, and the part which lies toward the internal portion and parallel with the sucker is 0.20 mm.

The shape and size of the tridents are so characteristic of all the specimens studied, that together with the general characteristics of the scolex and body, one can feel certain of its specific place in the genus.

The cuticula, as well as the parenchyma, is typical of all cestodes. The excretory system consists of two sets of tubules, two ascending and two descending, the latter uniting posteriorly and emptying into an excretory bladder.

The larvae were also found in the intestine of *Esox lucius* and were identical with the form found in the flesh of *Leucicthys tullibee* except that the former showed a trifle more advanced development.

The adult stage of *Triaenophorus robustus* was recovered from a single specimen of *Esox lucius* taken from Burntside Lake, July, 1927. The only entire worm found was 100 mm. long and 2.5 mm. wide in the widest part. Two pieces of this species were studied which were from individuals of considerable length. A posterior portion measuring 90 mm. long was made up of very mature proglottids. Each proglottid had a large swelling showing the expanded uterus filled with eggs. The other piece, also made up of mature proglottids, measured 65 mm. in length.

The adult cestode is white in color, the body showing no external segmentation (fig. 2). The cuticula, which is firm, is in numerous folds and creases, which begin immediately behind the posterior end of the scolex. These folds or creases do not in any way correspond to the internal segmentation. These contractions in the adult form are considerably stronger and deeper than those observed in the larval form. The average width of the body is more or less uniform, although, as in the case of the larva, the greatest width occurs in about the center of the body.

The scolex (fig. 2) is identical with the larva except that the bothria are much deeper and more prominent. The measurements of the scolices showed a variation from 0.92 mm. to 1.29 mm. in length. The width posteriorly varied from 0.92 mm. to 1.38 mm. The anterior part measured from 0.67 mm. to 0.92 mm. From studies made by others and from my own observations, it is clear that a large formidable scolex is very characteristic of this species.

Through the study of the internal structures one finds the most noticeable changes from the larva to the adult form. As in the case of the larva, the systems, with the exception of the reproductive system, are characteristic of all cestodes, so will not be taken up here.

### THE REPRODUCTIVE SYSTEM

In proglottids which are not fully matured the genital organs can be clearly made out. Each proglottid has a single genital apparatus which occupies most of the space between the dorsal and ventral transverse musculature. The genital openings are marginal on either side. In some cases the marginal openings were found in 3 or 4 proglottids on the same margin, then alternating to the opposite margin, so that while the pores may be on either side and alternating, the arrangement is very irregular. The uterine pore is ventral near the median line occurring on either side of the mid-line corresponding to the genital pores. It is always located away from the margin having the genital opening.

*The Male Organs*

The testes (fig. 3) are large nearly round bodies occupying all of the space not taken up by the other organs. They number approximately 100 to 110 in each proglottid. They are from 64$\mu$ to 72$\mu$ in diameter. The sperm cells are arranged in clusters within the testes, measuring from 19$\mu$ to 28$\mu$, with approximately 5 or 6 clusters within each testis. The vas efferentia could not be determined.

The cirrus sac (fig. 4) lies ventral to the nerve trunk occurring at either margin irregularly. At its opening it measures 10$\mu$. From here it goes in a rather straight course towards the dorsal side. In segments where the uterus is developed the cirrus sac is slightly more convoluted. It grows as it nears the vas deferens, having a width of from 96$\mu$ to 0.11 mm. in its widest part. In sections it appears as a heavy muscular body, the wall being from 3$\mu$ to 4$\mu$ in thickness. Around the outer covering of the sac, numerous elongated cells are attached, which have a large well defined nucleus. The cells appear glandular, but the function of these multitudinous cells was not determined. Very near the dorsal side and just below the dorsal transverse muscle layer, the cirrus sac proper terminates and a continuation known as the vas deferens in a series of convolutions continues.

Within the cirrus sac lies the cirrus, a muscular organ which is from 17$\mu$ to 21$\mu$ in thickness. Near the marginal opening the cirrus appears funnel shaped. The everted cirrus was never seen in any of the sections.

The position of the vas deferens (fig. 3) is usually median and dorsal to the ovary. It extends beyond the median line away from the cirrus sac and lies in numerous convolutions which occupy nearly all of the medullary parenchyma when the uterus is not fully developed. The posterior part of the vas deferens was not seen, but undoubtedly somewhere near the posterior extremity, the sperm cells are discharged into it from the vas efferentia.

*The Female Organs*

The vagina opens marginally with the cirrus sac, and ventral to it. Near its opening it is approximately 6$\mu$ in thickness, but widens out to a width of 19$\mu$ which width is more or less uniform for the remainder of its course. From its opening it extends parallel with the cirrus sac, ventral to it, for a distance of 0.32 mm., at which point it swings dorsally and crosses the cirrus sac. From here it passes towards the center of the proglottid, extending towards the ventral line, where it meets the oviduct and empties into it (fig. 3). At this point it measures 9$\mu$ in thickness. Just above the entrance to the oviduct, is the receptaculum seminis, which is very small, merely a slight expansion of the vagina.

The ovary (fig. 3), which is irregular in shape, lies ventral and close to the transverse muscle fibers. It appears in about the middle of the proglottid, yet its greatest mass is nearer the side where the marginal genital opening occurs. The largest ovary measured was 0.58 mm. in length and 0.25 mm. in its greatest width. The ovary is filled with ova of different stages of development. Near the oocapt the ova, which are ready to be discharged into it, are larger and more fully developed.

The oocapt (fig. 3) is an enlargement of the oviduct near the ovary, usually located towards the middle of the segment ventrally.

The oviduct (fig. 3), which is a continuation of the oocapt, is a tube measuring 8$\mu$ in width. As it leaves the ovary, it passes towards the side opposite the marginal for about 86$\mu$. Then it turns dorsally, passing through the shell gland, which lies near the ovary and is quite large. Posterior to the shell gland the yolk material from the vitelline follicles is discharged into the oviduct through a common vitelline duct. Leaving the shell gland the oviduct passes dorsally a short distance. From here it turns back towards the center of the proglottid where it finally empties into the uterus.

The uterus (fig. 4) has its origin in a weakly coiled tube, lying in the central portion of the proglottid, near the ventral line. As it grows and expands it crowds the other organs back until it occupies the entire segment, reaching from the dorsal transverse muscle to the ventral, a space measuring 0.33 mm. The uterus empties ventrally through a small opening about 19$\mu$ in diameter, occurring on either side of the median line, always away from the marginal genital opening.

The vitellaria (fig. 3) lie in one continuous row, surrounding the proglottid, lying dorsal to the inner longitudinal muscles and ventral to the spindle-shaped epithelial cells of the cortical layer. They are large deeply staining bodies, elliptical or round in shape. The diameter of the predominant type varies from 27$\mu$ to 57$\mu$. The vitellaria are follicular in structure, possessing tubules which are discharged into the two large tubes which finally unite, forming a reservoir which measures from 25$\mu$ to 51$\mu$. From here it narrows into a tube which empties into the oviduct just posterior to the point where the shell gland empties into it.

## PARTIAL LIFE HISTORY

From the works of other writers, as well as from my own observations, it is evident that *Triaenophorus robustus* undergoes its development first in a plankton form, probably a cyclops of some species. When the first intermediate host is eaten by the fish, the larvae migrate to the musculature and undergo a partial development there. The white fish (Tullibee) in whose flesh the Triaenophorus larvae were found encysted, is a 100% plankton feeder. Numerous stomachs of the white fish examined microscopically showed them to contain nothing but

entamostraca. Several species of cyclops were found, but no larvae of Triaenophorus were observed. The white fish are, in turn, eaten by *Esox lucius*. The scales from the white fish, as well as parts of them were found in the stomach. On one occasion an entire white fish measuring 4 inches long, was recovered from the stomach of an *Esox lucius*.

During the collecting of the material, no eggs from mature specimens were obtained, hence no feeding experiments with cyclops could be made. It is the hope of the writer that further research can be done on this form to complete the life history of the Triaenophorus which is identical with the European *Triaenophorus robustus* Olsson.

## SUMMARY

An intensive study has been made of material collected from eight lakes of northern Minnesota in the vicinity of Ely, Minnesota. A form of Triaenophorus identical with the European *Triaenophorus robustus* Olsson was found in *Leucicthys tullibee* and *Esox lucius*. The morphology of the larva and adult was worked out in detail, as well as a partial life history.

## References Cited

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Olsson, P. 1892.—Bidrag till skandinaviens helminthfauna. 2. Handl. K. Svensk. Vet. Akad., n.f. 25, 41 pp., 5 pl.

## List of Abbreviations

*b*—bothrium
*cs*—cirrus sac
*cw*—cyst wall
*e*—eggs
*l*—larva
*lm*—longitudinal muscles
*mt*—host muscular tissue
*o*—ovary
*oc*—oocapt
*od*—oviduct
*t*—testis
*tm*—transverse muscle
*u*—uterus
*v*—vitellaria
*va*—vagina
*vd*—vas deferens

## Explanation of Plate IV

Scale in figure 1 represents 1 mm., those in figures 2, 3 and 4 represent 0.3 mm.

Fig. 1.—Transverse section of cyst, showing larva within.

Fig. 2.—*Triaenophorus robustus* from *Esox lucius*, scolex of adult, toto.

Fig. 3.—Transverse section of mature segment.

Fig. 4.—Frontal section of mature segment, showing cirrus sac and uterus.

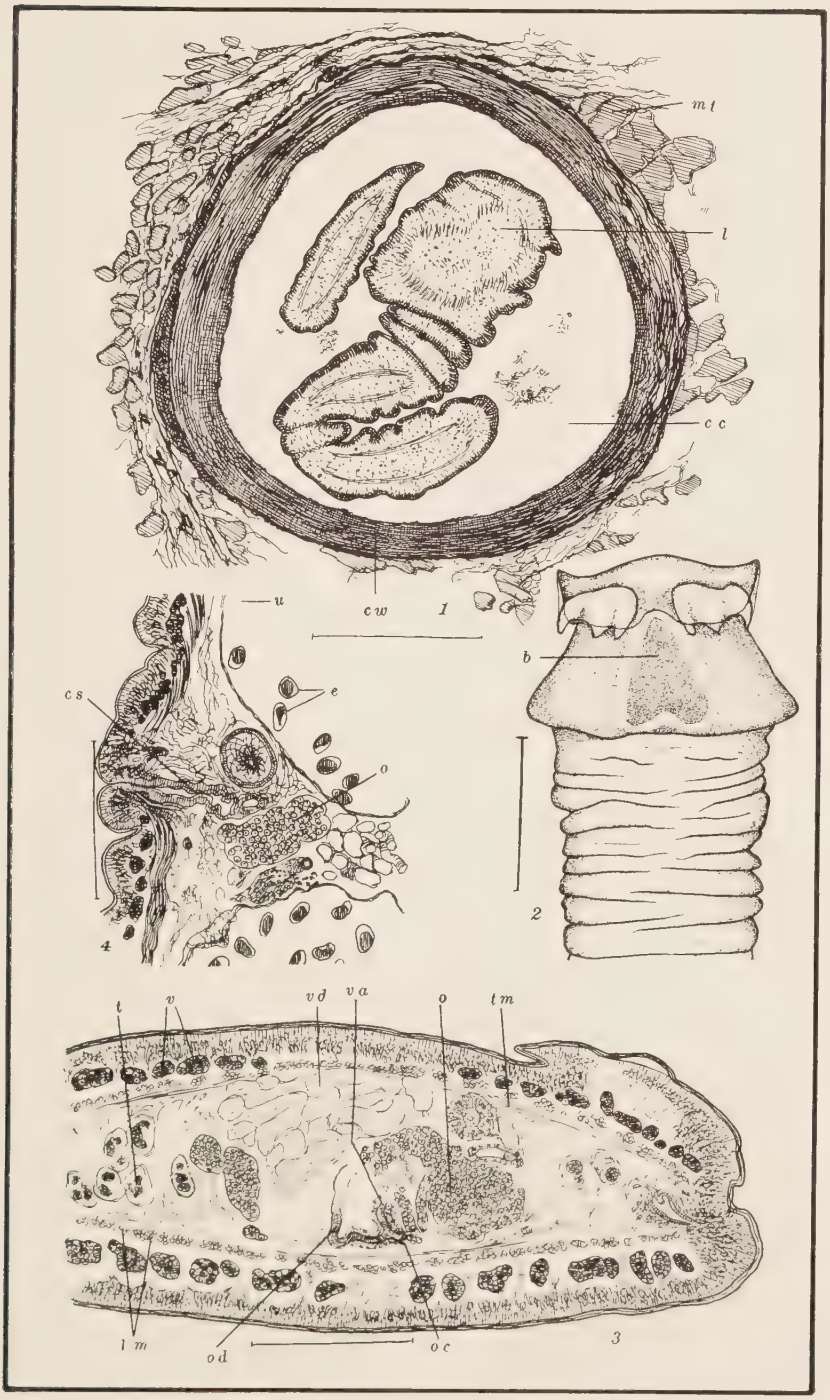


PLATE IV

# THE OVA-PARASITE RATIO FOR *ANCYLOSTOMA DUODENALE* AND *ASCARIS LUMBRICOIDES* *

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## I. OVA-PARASITE RATIO FOR *Ancylostoma duodenale*

Stoll (1922-23) devised a method for counting hookworm ova in feces and determined the relation between the number of ova per gram of excrement and the number of female worms, *Necator americanus,* harborbed by the host. This ratio varied from 44 eggs per gram per female worm on formed feces, to 25 eggs per female on mushy stools, to 12 eggs per gram per female worm on diarrhoeic stools. The same factors have since been found to hold for this parasite, by Sweet (1925) in Ceylon, and by Manalang (1927) in the Philippine Islands.

So far there is but little information of this relationship for the old world hookworm, *Ancylostoma duodenale,* the majority of observations having been made on mixed infections with *N. americanus.* Darling (1922) on the basis of a few comparative flotation egg counts and worm counts believed that *A. duodenale* produced a larger number of eggs than *N. americanus* and Sweet (1924) pointed out, largely on theoretical grounds, that the egg production of *A. duodenale* may be about five times as great as *N. americanus.* Cort, Grant and Stoll (1926) reported from a study of about fifty Chinese cases of mixed infections, three-fifths *A. duodenale* and two-fifths *N. americanus,* that the number of eggs per gram per female worm is about 200. This figure makes the egg output for *A. duodenale* about three times that found by Stoll (1923) in pure infections with *N. americanus* in Porto Rico. Soper (1927) from a statistical analysis of four Paraguayan hookworm cases with *N. americanus* and *A. duodenale* (three mixed infections and one pure Necator) concluded that the average egg output per day of *A. duodenale* is between two and two and one-half that of *N. americanus.*

The present study was made to gain definite knowledge on the ova-parasite ratio for *Ancylostoma duodenale,* when this parasite is the only hookworm present in a given population. This investigation was carried on in the laboratories of the Department of Parasitology of the Egyptian Government School of Medicine, Cairo, Egypt, with a field laboratory in

* This investigation was carried on by the Department of Comparative Pathology, Medical School and School of Public Health, Harvard University, with the cooperation of the International Health Division, Rockefeller Foundation. The work was done in the laboratories of the Department of Parasitology, Government School of Medicine, Cairo, Egypt.

the Government Hospital at Caliub, a village about twelve miles north of Cairo. Egypt is an ideal place to make such a view of the fact that there *A. duodenale* is the only hookworm known to infect man.

Through the cooperation with Ancylostoma sections of the Government Hospital at Caliub it was possible to secure patients who had applied for hookworm treatment. Upon admission the patient was assigned to a bed and kept in the ward until all observations were completed. Ova counts were made on each patient according to Stoll's recent modification of his technic (Stoll and Hauscheer, 1926) after which the patient was given a saline purge, and anthelmintic treatment on the morning following the purge. All patients were treated by Dr. M. Nazmi on individual prescription. The drugs used were carbon tetrachloride and oil of chenopodium. The average adult dosage used was 3 cc. of carbon tetrachloride plus 1.5 cc. oil of chenopodium followed by a saline purge. A saline purge was given the second day after treatment to insure a movement on the third day. Each patient was provided with an individual bed chamber in which he was instructed to pass all of his discharges. Both day and night nurses were constantly on duty to see that this measure was rigidly carried out. The stools were collected daily for three days and were carefully searched for worms by washing through brass sieves of 40 meshes to the inch. All the worm counts, as well as the egg counts were either made or checked by us. At the end of the third day the patient was instructed to return at the end of one month for reexamination.

It is with a sense of real pleasure for us to express our thanks for the courtesies and cooperation received during the course of this investigation from the Egyptian Department of Public Health which is under the general direction of Shahin Pasha. We are particularly indebted to Col. H. Marrian Perry, Director of the Public Health Laboratories, for his constant aid and active interest throughout the work. We are grateful to Dr. M. S. Abaza of the Ancylostoma Section for his cooperation and to the Research Section of the Public Health Laboratories for the loan of trained assistants whose diligence was an important factor in the success of the work, and to Dr. M. Helmy and Dr. M. Nazmi of the Department of Parasitology, Government Medical School, and M. Zalah, also of the Research Section for assistance in these and other research projects carried on at this time. Special thanks are due the Staff of the Caliub Hospital, particularly the members of the Ancylostoma-Bilharzia Sections, for their cooperation and accommodations. Special mention should be made of the aid received from Miss Dorothy Weeks in assisting in the organization of the data for publication.

Seventy-four hospital cases were the basis of study. In each case the ova count and female worm count were available at the time of the

first treatment, and the ova count a month later. The cases were divided into two groups with three parts each.

Group 1. Cases negative one month later:
Character of stool at time of first examination,
1 Formed (F—)
2 Soft Formed (SF—)
3 Mushy (M—)

Group 2. Cases positive one month later:
Character of stool at time of first examination,
1 Formed (F+)
2 Soft Formed (SF+)
3 Mushy (M+)

In the majority of cases the ova count was taken at least twice, the average ova count being used in all computations. The number of ova per female worm was computed for each case. The observations of each part under Group 1 were considered separately. In this group the relation was found to be about the same for the (F—) and the (SF—) groups and that twice as many female worms were necessary to produce the same number of ova in a mushy stool as in a formed stool. Assuming these relations to be the same in the second group, corrections were made for the ova count at the time of the second examination, and the relation of ova and female worm count determined after such corrections were made. To illustrate, case No. 66 at the time of the first examination, the character of the stool was formed, 155 ova were counted, 73 female worms were recovered after treatment. At the time of the second examination in a mushy stool 16 ova were counted. Assuming no additional infection occurred between the time of the first and second examination the 73 female worms must have then produced 123 ova (155—2(16) ova) at the time of the first examination. The relation of ova and female worm count was also determined by considering other combinations of the various parts under the two groups, as well as taking the total number of hospital cases as a whole, after all had been brought up to the formed basis.

Throughout this analysis, the statistical methods used are the same as those used by Stoll (Cort, Stoll and Grant, 1926: 24-28). Throughout the whole study, the computations were carried to two decimal places except for the coefficient of variations and their probable errors. Therefore except for the mushy stools and (SF+) all computations of the means have introduced an error approximating 1%. Inasmuch as the probable errors of the means are considerably greater than 1%, and the values for the corresponding standard deviations and coefficients of variations are relatively higher, the error thus introduced is considered negligible.

From table 1 the constant expressing the number of ova per female worm per 0.075 cc. of the 1 : 15 fecal suspension for (F), (SF), ($M_F$) stools varied from 1.07 to 1.41 except for (SF—). The value 1.19 appears twice, and each time with the series containing the largest number of cases. This value 1.19 lies well within the range of the probable value of the other constants when the probable errors are considered together with the value of the mean, except for the series (SF±). The probable errors of the mean 1.19 are small in comparison with the majority of the probable errors of the means. Although the standard deviations and coefficients of variation are relatively high, they group themselves, the same values repeating in the various series. This is felt to be significant, and gives confidence in accepting the value 1.19 ova per female worm

TABLE 1.—*Constants for Ancylostoma*

| Character of Stool | Number of Cases | Mean of Ova per Female Worm in 0.075 Cc. of 1:15 Fecal Suspension | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|
| (F—) | 16 | 1.07 ± .11 | .64 ± .08 | 60 ± 10 |
| (SF—) | 11 | 1.39 ± .18 | .87 ± .13 | 63 ± 12 |
| (M—) | 10 | .66 ± .12 | .57 ± .08 | 86 ± 20 |
| (F+) | 22 | 1.27 ± .12 | .81 ± .08 | 64 ± 9 |
| (SF+) | 7 | .68 ± .07 | .26 ± .03 | 38 ± 8 |
| (M+) | 8 | .56 ± .05 | .21 ± .02 | 38 ± 7 |
| (F—, SF—) | 27 | 1.20 ± .10 | .76 ± .07 | 63 ± 8 |
| (F+, SF+) | 29 | 1.13 ± .09 | .76 ± .07 | 67 ± 8 |
| (F±) | 38 | 1.19 ± .08 | .76 ± .06 | 64 ± 7 |
| (SF±) | 18 | 1.11 ± .01 | .78 ± .09 | 70 ± 11 |
| (M±) | 18 | .61 ± .07 | .45 ± .05 | 74 ± 12 |
| *($M_F$ —) | 10 | 1.41 ± .23 | 1.10 ± .16 | 78 ± 17 |
| *($M_F$ +) | 8 | 1.11 ± .10 | .41 ± .07 | 37 ± 7 |
| *($M_F$ ±) | 18 | 1.28 ± .14 | .87 ± .14 | 68 ± 11 |
| All cases | 74 | 1.19 ± .06 | .79 ± .04 | 66 ± 5 |

* Mushy stools brought up to formed basis ($M^F$).

per 0.075 cc. of the 1 : 15 fecal suspension for formed stools. The high correlation coefficient .91±.01 also confirms this point of view.

To express the number of ova per cc. of stool, the number of ova per 0.075 cc. from the 1 : 15 suspension is multiplied by 200. The number of ova per cc. of stool per female worm would then be about 238 for formed stools. This figure agrees with the findings of Stoll in China where 200 ova per cc. of stool per female worm was the figure determined for infections with *Ancylostoma* and *Necator,* three-fifths *A. duodenale,* and two-fifths *N. americanus.* This figure of 238 is then in accordance with the belief that *Ancylostoma* is the greater ova producer of the two species of hookworms of man.

Table 2 shows the comparison of the actual and estimated numbers of male and female worms for all cases, with ova counts reduced to the formed basis. These data also indicate that the male and female

worms occur in equal numbers. The estimated number of male and female worms is twice the estimated number of female worms. The estimated number of female worms was determined by dividing the hookworm ova per 0.075 cc. of fecal suspension by 1.19.

### II. THE OVA-PARASITE RATIO FOR ASCARIS LUMBRICOIDES

Although it is generally known that the female of *A. lumbricoides* produces a very great number of eggs, there is but little accurate information on the matter. Cram (1925) estimated that a single female of that species may contain as high as 27,000,000 ova at one time. Brown and Cort (1927) calculated that a single female of *A. lumbricoides* produced about 2,000 ova per gram feces. This figure was based on a study of two cases in Panama and was presented as a tentative ova-parasite ratio for the human *Ascaris* with the hope that it would be checked by others having a larger number of cases. Along with our Egyptian *Ancylostoma* studies, we were able to make similar correlation studies on *Ascaris lumbricoides* as many of our patients included in that study were also infected with this parasite.

TABLE 2.—*Comparison of Actual and Estimated Number of Male and Female Worms for All Cases. All Ova Counts on the Basis of Formed Stools*

| | Hookworm Ova per 0.075 Cc. of Fecal Suspension | Actual Number of Female Worms Collected | Estimated Number of Female Worms | Actual Number of Male and Female Worms Collected | Estimated Number of Male and Female Worms |
|---|---|---|---|---|---|
| All case — | 927.1 | 1037 | 782 | 1879 | 1564 |
| All case + | 2918.53 | 2955 | 2450 | 5853 | 4900 |
| Total | 3845.63 | 3992 | 3232 | 7732 | 6464 |

Twenty-seven cases were the basis for our study. In each case the ova count and female worm count were available at the time of the first treatment, and the ova count a month later. The cases were first divided into three groups. All cases used in this study were negative by Willis' method at the time of the second examination.

Character of stool at time of first examination.
1 Formed (F—)
2 Soft Formed (SF—)
3 Mushy (M—)

The statistical study was carried out in the same manner as with the Ancylostoma study. The number of ova per female worm was computed for each case, and each group was considered separately. The relation was found to be about the same for the (SF—) and (M—) series, and that three times as many female worms were necessary to produce the same number of ova in these stools as in a formed stool. The relation of ova and female worm count was also determined by

considering the total number of hospital cases as a whole, after all had been brought up to the "formed basis." Throughout this analysis, the statistical methods used are the same as those used in the Ancylostoma study.

From table 3 the constant expressing the number of ova per female worm per 0.075 cc. of the 1:15 fecal suspension for all series on the formed basis varies from 11.61 to 15.22. The calue 13 appears three times and each time with the series containing the largest number of cases. The value of 13.5 (the one determined from the series of all the cases) was selected. This value 13.5 lies well within the range

TABLE 3.—*Constants for Ascaris Study*

| Number of Cases | Kind of Stool | Mean Ova per FW per 0.075 Cc. | SD | CV |
|---|---|---|---|---|
| 13 | (F —) | 13.4 ± 1.4 | 7.5 ± 1.0 | 56 ± 10 |
| 8 | (SF —) | 5.1 ± .8 | 3.3 ± .6 | 64 ± 15 |
| 6 | (M —) | 3.9 ± .4 | 1.6 ± .3 | 40 ± 9 |
| 14 | (SF —, M —) | 4.6 ± .5 | 2.7 ± .3 | 59 ± 10 |
| 8 | *($SF_F$ —) | 15.2 ± 2.3 | 9.8 ± 1.6 | 64 ± 15 |
| 6 | *($M_F$ —) | 11.6 ± 1.3 | 4.7 ± .9 | 40 ± 9 |
| 14 | *($SF_F$ —, $M_F$ —) | 13.7 ± 1.5 | 8.0 ± 1.0 | 60 ± 10 |
| 27 | All case | 13.5 ± 1.0 | 7.9 ± .7 | 59 ± 7 |

* Mushy and soft formed stools brought up to formed basis.

TABLE 4.—*Comparison of Actual and Estimated Numbers of Male and Female Worms for All Ascaris Cases Negative at the Time of Second Examination. All Ova Counts Are on the Basis of Formed Stools*

| | Ascaris Ova per 0.075 Cc. of Fecal Suspension | Actual Number of Female Worms Collected | Estimated Number of Female Worms | Actual Number of Male and Female Worms Collected | Estimated Number of Male and Female Worms |
|---|---|---|---|---|---|
| All cases | 2026 | 174 | 152 | 300 | 304 |

of the probable values of the other constants, when the probable errors are considered together with the value of the mean, except for the series ($M_F$—). Therefore the value 13.5 is felt to more nearly express the number of ova per female worm per 0.075 cc. of the 1:15 fecal suspension for formed stools. The correlation coefficient of the ova counted and female worms recovered for all cases expressed as formed stools was found to be .74 ± .08. To express the number of ova per cc. of stool, the number of ova per 0.075 cc. from the 1:15 fecal suspension is multiplied by 200. The number of ova per cc. of stool per female worm would then be about 2,700 for formed stools. Our figure therefore substantiated that of Brown and Cort and, although somewhat greater, gives, perhaps, a more exact estimation in view of the greater number of cases included in our series.

Table 4 shows the comparison of the actual and estimated numbers of male and female worms for all Ascaris cases. All ova counts are brought up to the basis of formed stools. The data also indicate that the male and female worms occur in equal numbers. The estimated number of male and female worms would then be twice the estimated number of female worms. The estimated number of female worms was determined by dividing the Ascaris ova per 0.075 cc. of fecal suspension by 13.5.

SUMMARY

1. An intensive study of 74 Egyptian hookworm cases, *Ancylostoma duodenale,* has shown that the female worm produces about 238 ova per cc. formed feces. The factor 1.19 may be used to estimate the number of females present when using the small drop, 0.075 cc., according to Stoll's method.

2. A similar study of 27 Ascaris cases, *Ascaris lumbricoides,* has shown that the female worm produces about 2,700 ova per cc. formed feces. With this parasite the factor 13.5 was determined for computing the number of female worms harbored by the host from the number of ova present in the stool, Stoll's method.

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# STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) NO. X *NEASCUS BULBOGLOSSA* (VAN HAITSMA) *

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A new species of strigeid metacercaria, *Neascus bulboglossa* (Van Haitsma) determined by an unpublished feeding experiment performed by Mr. Van Haitsma to be the larva of *Crassiphiala bulboglossa* Van Haitsma, was found encysted in *Perca flavescens* Mitchill taken from the mouth of Bessey Creek, a tributary of Douglas Lake, in Cheboygan County, Michigan, during the summer of 1927. The adult trematode is parasitic in the gut of *Ceryle alcyon* Linnaeus in the vicinity of Douglas Lake (Van Haitsma 1925: 122). Although the parasites occur, chiefly, widely distributed in the integument, a few were found in the myotomes and on the gill arches.

Fifteen out of 16 yellow perch, varying in length from 67 mm. to 133 mm. (average 90 mm.), from the mouth of Bessey Creek, were parasitized with from 2 to 530 (average 75) specimens of the present species. The parasite was not found in any of a considerable number of yellow perch collected from other parts of Douglas Lake. A worm, similar to, and perhaps identical with, the present species was briefly described by Cooper (1915: 202; Pl. III, figs. 25, 26) as *Cercaria* sp. from black-pigmented cysts in a minnow.

Most of the observations herein recorded were made on living specimens flattened by cover-glass pressure. For whole mounts, specimens were stained, regressively with a dilute solution of Ehrlich's acid hematoxylin in 70% alcohol. For sectioning, specimens were stained *in toto* in Ehrlich's acid hematoxylin, decolorized on the slide and counterstained with eosin. The present species resembles *Neascus ambloplitis* Hughes more closely than any other species and will be described in comparison with it.

The cyst, like that of *N. ambloplitis* (Hughes 1927: 249) comprises 2 parts, an outer lamellar, syndesmous, black-pigmented portion of host origin and an inner non-cellular thin tough resilient hyalin layer of parasite origin. In 10 specimens, the outer cyst, nearly spherical in shape, varied in diameter from 0.36 mm. to 0.68 mm. (average 0.47 mm). The inner cyst, which closely invests the parasite and can hardly be removed without injury to it, opens readily in a warm bath of com-

* Contribution from the Biological Station and from the Department of Zoölogy, University of Michigan. This is No. X of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

mercial trypsin dissolved in a 0.5% aqueous solution of sodium bicarbonate. As in *N. ambloplitis* the anterior end of the parasite is the first to emerge from the inner cyst. Correlated with the fact that the whole anterior end of the cyst is ruptured this form emerges from the cyst much more readily than *N. ambloplitis.* The inner cyst (Fig. 5), smaller and relatively broader than in *N. ambloplitis,* is somewhat egg-shaped, truncate anteriorly. In *N. ambloplitis* the inner cyst (Fig. 6) is truncate posteriorly. Six inner cysts, containing living parasites and under cover-glass pressure, varied in length from 0.18 mm. to 0.22 mm. (average 0.2 mm.) and in width from 0.14 mm. to 0.18 mm. (average 0.16 mm.).

Although the animal is somewhat folded in the cyst, as is true of *N. ambloplitis,* its principal organs are distinguishable. A constriction (Fig. 2) divides the form of the parasite into well defined fore- and hind-body. Unlike *N. ambloplitis,* the long axis of the freed specimen is not flexed in the region of the constriction (Fig. 3). Freed from the cyst, the parasite is very active; its movements consist of alternately lengthening and shortening the body. Correlated with the absence of an acetabulum, the leech-like movement of *N. ambloplitis* and some other strigeid metacercariae does not occur. In the most extended condition of the body the constriction between the two body regions is smoothed out and the long and slender hind-body is not perceptibly delimited from the swollen fore-body (Fig. 3). In four living specimens, under cover-glass pressure, each in a state of muscular relaxation, the length of the body was 0.34 mm., 0.40 mm., 0.31 mm. and 0.36 mm. respectively. Five mounted specimens varied in length from 0.175 to 0.448 mm. (average 0.27 mm.). *Neascus bulboglossa* is much smaller than *N. ambloplitis.*

The fore-body is deeply cup-shaped. The swollen aspect of the fore-body, even in its most extended condition correlates with the fact that its cup-shaped concavity contains the extraordinarily large hold-fast organ. The surfaces of the fore-body and of the hold-fast organ inside and out are covered quincuncially with minute spines. In four living specimens, under cover-glass pressure, the length of the fore-body was 0.167 mm., 0.182 mm., 0.196 mm. and 0.218 mm. and the width 0.174 mm., 0.17 mm., 0.125 mm. and 0.145 mm. respectively. In five mounted specimens the length of the fore-body varied from 0.112 mm. to 0.193 mm. (average 0.14 mm.) and in width from 0.084 mm. to 0.109 mm. (average 0.096 mm.). In a mounted specimen the thickness of the fore-body was 0.105 mm. In *N. ambloplitis* the fore-body is not so deeply cup-shaped, the ventral lip being relatively much shorter than in the present species.

The hind-body, conical when relaxed, elongated and cylindrical when extended with the pointed posterior end curved retrorsely dorsad (Fig. 3), is less well developed than in *N. ambloplitis.* In four living

specimens under cover-glass pressure it was 0.138 mm., 0.218 mm., 0.16 mm. and 0.145 mm. long and 0.125 mm., 0.087 mm., 0.095 mm. and 0.087 mm. wide respectively. In five mounted specimens the length of the hind-body varied from 0.063 mm. to 0.158 mm. (average 0.128 mm.) and in width from 0.024 mm. to 0.07 mm. (average 0.055 mm.).

The hold-fast organ (Fig. 1, *hf*) is a large bulbous heavily staining structure attached in the usual position by a slender longitudinally elongate pedicle. Its ventral surface is marked by a deep median furrow, the ends of which are shortly bifurcate. In four living specimens under cover-glass pressure the length of the hold-fast organ was 0.111 mm., 0.111 mm., 0.084 mm., and 0.105 mm. respectively.

The relatively large spheroidal oral sucker is ventro-terminal in position. Its diameter in four living specimens under cover-glass pressure, was 0.039 mm., 0.042 mm., 0.039 mm. and 0.045 mm., and in five mounted specimens 0.031 mm., 0.032 mm., 0.035 mm., 0.038 mm., and 0.039 mm. respectively. Ridges on the external surface of the oral sucker such as were described for *N. ambloplitis* (Hughes 1927: 250) were not observed. The relatively large pharynx is a little smaller than the oral sucker. In two living specimens under cover-glass pressure its diameter was 0.021 mm. and 0.024 mm. respectively. According to Van Haitsma (1925: 123) the pharynx in the adult is nearly equal in size to the oral sucker. The very long esophagus bifurcates just in front of the hold-fast organ. The intestinal crura diverge at an obtuse angle about the base of the hold-fast organ, converging a little as they enter the hind-body and extend to the tip of the latter where they terminate blindly (Fig. 2).

Fundaments of reproductive organs, a hold-fast gland and a bursa copulatrix are wanting. The nervous system was not studied. Subcuticular layers of circular and longitudinal muscle fibers are present in both fore- and hind-bodies.

The excretory system of the present species closely resembles that of *N. ambloplitis* (Hughes 1927: 251) and as far as applicable the same terminology is used in the present description. The excretory system of the holostome larva comprises two parts, the primary excretory apparatus consisting of flame cells and associated tubules and the reserve bladder (Faust 1922: 80 and Hughes 1927: 253). As in *N. ambloplitis* the primary excretory apparatus is greatly obscured by the thickness of the animal and the excessive development of the reserve bladder. A few scattered flame cells and tubules were observed but not in sufficient detail to give any definite idea of their arrangement. The excretory system was studied both in living specimens and in sections. The former method was much more fruitful since in preserved material many of the finer details are lost. Sections were, however, indispensable for the study of certain larger features.

The reserve bladder, as in *N. ambloplitis,* comprises an intricate network of large and small vessels, the arrangement of which, although variable in details, shows a high degree of regularity in general plan. These vessels contain a limpid transparent fluid, in which float yellowish, highly refractive, calcareous granules of various sizes. This fluid with the granules contained surges about from one part of the body to another in response to muscular movements and now and then some is discharged from the excretory pore.

The principal vessels of the reserve bladder are as follows: The median dorsal vessel (Fig. 1, *m.*) extends from the region dorsal to the pharynx to about the end of the anterior fourth of the hind-body. In the postero-dorsal part of the hold-fast organ this vessel divides to form a ring about a small island (*hi*). A pair of vessels (*v*) connected with the median dorsal vessel before and behind the hold-fast organ encircle its base. These vessels are connected with the median dorsal vessel by a coarse network of anastomoses (*hn*). Anterior to the hold-fast organ the median dorsal vessel is connected by 4 pairs of transverse commissural vessels (*t*) with a pair of primary lateral vessels (*p*). *Neascus bulboglossa* differs from *N. ambloplitis* in that it has 4 instead of 7 pairs of transverse commissural vessels and that definite intra- and extra-lateral vessels are wanting. The primary lateral vessels are connected with a closed marginal vessel (*mc*), which is located in the lip of the cup-shaped concavity of the fore-body, by an irregular network of small anastomoses (*n*). This network is most intensive in the lateral parts of the ventral lip of the fore-body. The primary lateral vessels unite at the juncture of the two body regions to form a short median ventral vessel (*mv*). The arrangement of the vessels in the hind-body is the same as described for the adult by Van Haitsma (1925: 124). The median dorsal vessel bifurcates forming a pair of dorso-lateral vessels (*d*). The median ventral vessel also bifurcates, at a point slightly anterior to the place of division of the median dorsal vessel, forming a pair of ventro-lateral vessels. The ventro- and dorso-lateral vessels on either side anastomose in the posterior third of the hind-body forming a pair of postero-lateral vessels (*pv*). These in turn unite to form a short median excretory duct (*e*) which debouches through the terminal excretory pore (*ep*). Conspicuous semicircular commissures in the hind-body and a muscular excretory bladder are wanting, differing in these respects from *N. ambloplitis.* I was not able to find a connection between the median dorsal and median ventral vessel in the anterior part of the hind-body, such as Van Haitsma (1925: 24) reports for the adult, but such a difference might be due to differences in degree of development. Such a connection occurs in *N. ambloplitis* (Hughes 1927: 253).

## DISCUSSION

The present species has all of the characters diagnostic of the larval group Neascus as given by Hughes (1927: 259), having fore- and

hind-bodies well developed and distinctly demarcated, no lateral sucking-cups, fore-body leaf-like, hold-fast organ well developed, reserve bladder highly developed, the smaller branches of which are anastomoses, calcareous granules free in the circumambient fluids, and being encysted. It differs, however, from all other members of the group known to me with respect to (1) the degree of cupping of the fore-body, (2) the large size of the pharynx, (3) the great length of the esophagus, (4) the excessive development of the hold-fast organ, (5) the absence of an acetabulum, (6) the lack of rudiments of reproductive organs and a bursa copulatrix and (7) the peculiar curving of the posterior end of the hind-body, when that part of the body is elongated, a condition which obtains in both living and preserved specimens. It must be acknowledged, however, that in regard to some of these points *Neascus musculicola* (Waldenburg) and *Neascus brevicaudatus* (von Nordmann) have not been carefully studied. In regard to the large size of the pharynx, the excessive development of the hold-fast organ, and the absence of an acetabulum *N. bulboglossa* is unique among the strigeid metacercariae heretofore described. A comparative study of the known species of the larval group Neascus is being presented elsewhere.

I wish here to express my grateful appreciation to Professor George R. La Rue of the Zoölogy Department and Director of the Biological Station, University of Michigan, by whom this study was suggested and under whom it has been conducted. I am also indebted to Mr. J. P. Van Haitsma of the Department of Organic Science, Calvin College, for many helpful suggestions.

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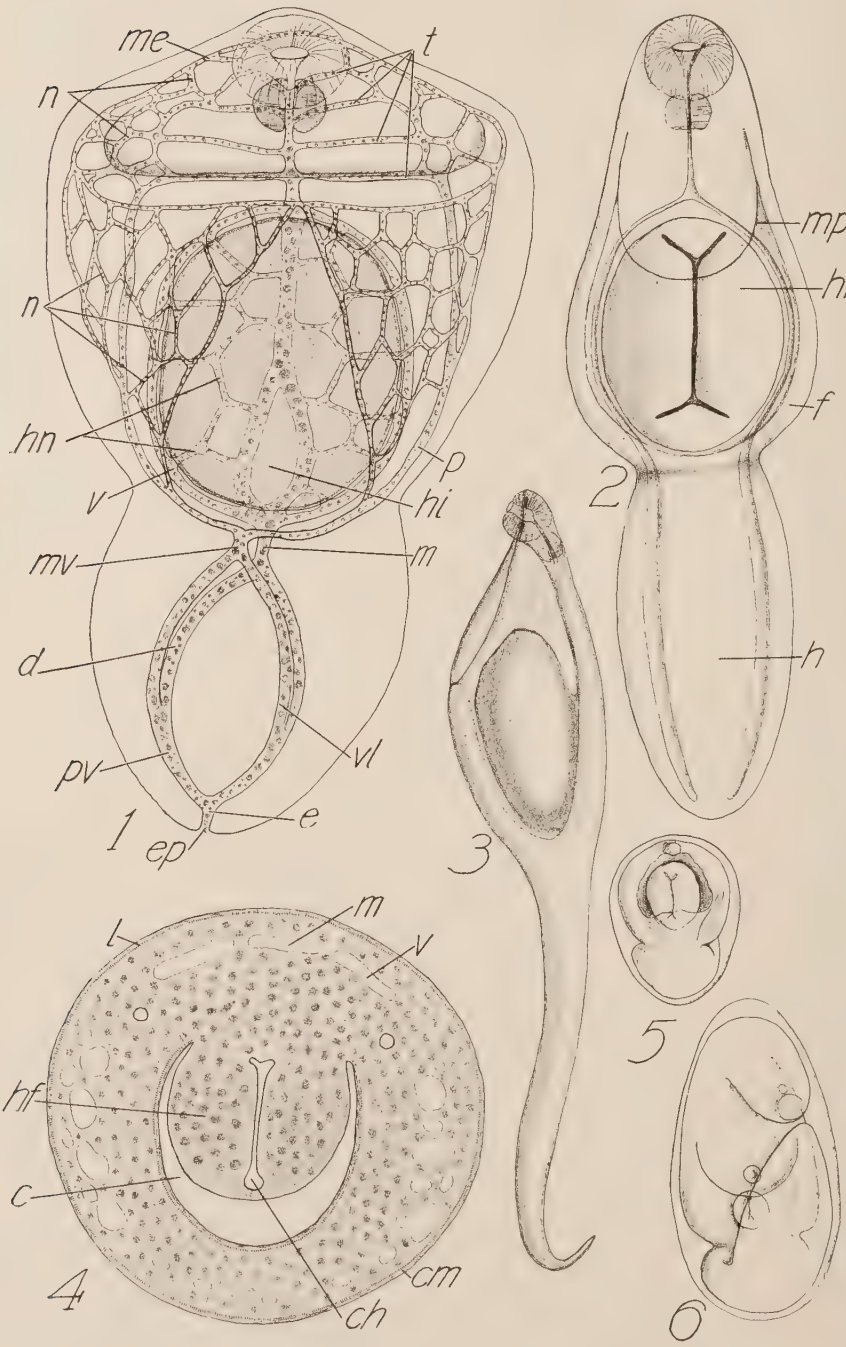


PLATE V

Abbreviations Used

*c*—concavity of fore-body
*ch*—cavity of hold-fast organ
*cm*—circular muscle fibers
*d*—dorso-lateral excretory vessel
*e*—excretory duct
*ep*—excretory pore
*f*—fore-body
*h*—hind-body
*hf*—hold-fast organ
*hi*—island in median dorsal excretory vessel in base of hold-fast organ
*hn*—network of excretory vessels in base of hold-fast organ
*l*—longitudinal muscle fibers
*m*—median dorsal excretory vessel
*mp*—margin of posterior lip of fore-body
*me*—marginal excretory vessel
*mv*—median ventral excretory vessel
*n*—network of small excretory vessels connecting primary lateral excretory vessel with marginal excretory vessel
*p*—primary lateral excretory vessel
*pv*—postero-lateral excretory vessel
*t*—transverse commissural excretory vessels
*v*—excretory vessel encircling base of hold-fast organ
*vl*—ventro-lateral excretory vessel

Explanation of Plate V

Figs. 1, 2, 3, 5 and 6 were drawn, free-hand, from living specimens. Figs. 5 and 6 were drawn to the same scale. Figs. 1, 2 and 3 were drawn to different scales and represent different states of contraction. Except Fig. 6 all represent *Neascus bulboglossa*.

Fig. 1.—Ventral view in much shortened condition, showing principal vessels of so-called reserve bladder.

Fig. 2.—Ventral view in state of muscular relaxation, showing digestive system and external features of body.

Fig. 3.—Side view in much elongated condition showing peculiar cup-shaped conformation of fore-body and large hold-fast organ.

Fig. 4.—Cross-section through region of hold-fast organ. × 621.5 approx.

Fig. 5.—Inner cyst.

Fig. 6.—Inner cyst of *Neascus ambloplitis* drawn to the same scale as Fig. 5.

# STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) NO. XI *NEASCUS PTYCHOCHEILUS* (FAUST) *

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AND
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During the summers of 1926 and 1927 a strigeid metacercaria, belonging to the larval group Neascus Hughes and apparently identical with *Cercaria ptychocheilus* Faust was found encysted in *Notropis deliciosus stramineus* Cope at Douglas Lake in Cheboygan County, Michigan. Since the descriptions by Faust (1917: 110 and 1917a: 66) are brief and in certain respects erroneous we are redescribing the species. An examination of 25 *Notropis deliciosus stramineus,* varying in length from 40 mm. to 65 mm. (average 48 mm.), revealed 22 parasitized with from 1 to 257 (average 32) cysts of the present species. We have examined for purposes of comparison two cotype specimens † of *Cercaria ptychocheilus* taken from *Ptychocheilus oregonensis* Richardson caught in Montana in April, 1915. In *P. oregonensis* Faust (1917: 110) found this parasite "in very large numbers (several thousands)." Our specimens were found in cysts attached to the peritoneum of the body cavity and viscera and in cysts free in the ovaries. Faust found his specimens in mesenteric cysts.

*Neascus ptychocheilus* closely resembles *N. van-cleavei* (Agersborg) as described by Hughes (1928) and the method of study outlined in that paper has been used here. For staining Ehrlich's acid hematoxylin was used. The cotype specimens, also stained with Ehrlich's acid hematoxylin, closely resemble our preparations.

In as much as the morphology of the present species is in many respects identical with that of *Neascus van-cleavei* we deem it necessary to describe in detail only those parts wherein the two species differ. When feasible we simply state that the structure under consideration is similar to that of *N. van-cleavei* as described by Hughes (1928) and avoid a repetition of description. In regard to the structure of the cyst and the structure and position of the oral sucker, acetabulum, holdfast organ and bursa copulatrix, the two species are quite alike.

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* Contribution from the Biological Station and from the Department of Zoölogy, University of Michigan. This is No. XI of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

† These specimens, mounted *in toto* on one slide, were submitted to the Department of Zoölogy of the University of Illinois by Dr. Faust with his doctor's thesis. This slide has been kindly loaned, for the sake of this study, to Professor George R. La Rue by Professor Henry B. Ward.

The cyst (Fig. 1) is often many times as large as the parasite and generally yellowish in color. Eleven cysts containing living parasites and not under pressure varied in length from 0.675 mm. to 0.9 mm. (average 0.75 mm.) and in width from 0.4 to 0.63 mm. (average 0.49 mm.).

The body of the parasite consists of the usual parts, the fore- and hind-bodies, which, however, are not distinctly set apart as in *Neascus van-cleavei*. The constriction (Fig. 4) is shallow or wanting. In side view the fore-body is wedge-shaped, thickened posteriorly where it joins the short rounded hind-body. The fore-body lacks a distinct postero-ventral lip, the concavity being very shallow or wanting. The hind-body is relatively much shorter and broader than in *N. van-cleavei*. In dorsal or ventral view, the outline of the body in living specimens is oblong (Fig. 4) and in preserved specimens nearly circular to broadly ovate, the separation of the body into two regions being scarcely discernible in either case. The anterior end in both living and preserved

*Measurements in Millimeters of Living* Neascus ptychocheilus, *Freed from Cysts and Subjected to Cover-Glass Pressure*

| Number of specimen | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Length | Extended.. | 0.797 | 0.747 | 0.764 | 0.847 | 0.789 | 0.730 | 0.813 | 0.772 | 0.706 | 0.739 | 0.770 |
| | At rest..... | 0.697 | 0.697 | 0.697 | 0.780 | 0.714 | 0.664 | 0.730 | 0.664 | 0.647 | 0.674 | 0.694 |
| | Contracted | 0.623 | 0.598 | 0.631 | 0.664 | 0.647 | 0.564 | 0.631 | 0.581 | 0.548 | 0.598 | 0.608 |
| Width | Extended.. | 0.390 | 0.382 | 0.332 | 0.415 | 0.399 | 0.374 | 0.398 | 0.374 | 0.349 | 0.357 | 0.377 |
| | At rest..... | 0.315 | 0.298 | 0.299 | 0.315 | 0.307 | 0.282 | 0.332 | 0.291 | 0.291 | 0.299 | 0.303 |
| | Contracted | 0.252 | 0.249 | 0.282 | 0.274 | 0.266 | 0.257 | 0.257 | 0.245 | 0.249 | 0.254 | 0.259 |

specimens is notched in the region of the oral sucker (Fig. 4). The entire surface of the fore-body is covered quincuncially with minute spines.

Due to the fact that the animals were almost constantly in motion it was very difficult to make satisfactory measurements. In the course of its movements the body of the parasite alternately lengthens and shortens, becoming at the same time narrow and broad respectively. We endeavored to measure the parasites in their most extended and contracted shapes as well as in a condition of muscular relaxation. Measurements of 10 living specimens free from their cysts and under cover-glass pressure are presented in the accompanying table. Ten mounted specimens varied in length from 0.211 mm. to 0.317 mm. (average 0.259 mm.) and in width from 0.211 mm. to 0.268 mm. (average 0.233 mm.).

The activity of our specimens was similar to that of *N. van-cleavei*. We did not observe the "measuring-worm movement" reported by Faust (1917a: 67). The behavior of the tissues in fixation is also similar to that of *N. van-cleavei*. The hold-fast organ (Fig. 4 *hf*) in 10 living specimens under cover-glass pressure varied in length from 0.076 mm.

to 0.088 mm. (average 0.082 mm.) and in width from 0.096 mm. to 0.106 mm. (average 0.1 mm.) and, in 10 specimens mounted *in toto,* it varied in length from 0.024 mm. to 0.063 mm. (average 0.043 mm.) and in width from 0.063 mm. to 0.105 mm. (average 0.795 mm.). Faust (1917a: 67) interpreted the hold-fast organ to be the acetabulum. The transversely elongate hold-fast gland was regarded by Faust (1917a: Pl. IV, fig. 49) as an ovary.

The oral sucker in 10 living specimens subjected to cover-glass pressure varied in length from 0.028 mm. to 0.037 mm. (average 0.033 mm.) and in width from 0.028 mm. to 0.042 mm. (average 0.036 mm.). In five mounted specimens it varied in length from 0.01 mm. to 0.021 mm. (average 0.015 mm.). The acetabulum was regarded by Faust (1917a: 67) to be a "primitive genital pore." In 10 living specimens under cover-glass pressure the acetabulum varied in length from 0.035 mm. to 0.042 mm. (average 0.039 mm.) and in width from 0.035 mm. to 0.045 mm. (average 0.04 mm.). In mounted specimens the acetabulum is scarcely larger than the oral sucker, whereas in mounted specimens of *N. van-cleavei,* the acetabulum is more than twice as large as the oral sucker.

The bursa copulatrix in 7 specimens mounted *in toto* varied in length from 0.035 mm. to 0.053 mm. (average 0.044 mm.) and in width from 0.028 mm. to 0.035 mm. (average 0.034 mm.). The structures interpreted by Faust (1917a: Pl. IV, fig. 49) as genital atrium, oötype and oviduct are parts of the bursa copulatrix.

In 10 living specimens under cover-glass pressure the pharynx varied in length from 0.028 mm. to 0.04 mm. (average 0.035 mm.) and in width from 0.012 mm. to 0.019 mm. (average 0.017 mm.). The digestive system (Figs. 2, 3, 4, *in*), nervous system (Figs. 3, 4, *an, pn, s*), reproductive system (Figs. 2, 4, *r*) and musculature (Figs. 2, 3, 4, *cm, im, o*) are essentially the same as in *N. van-cleavei.* Fundaments of vitellaria are not found in the present species.

The "reserve bladder" (Fig. 5) differs from that of *N. van-cleavei* in only a few minor details. There are usually three instead of four pairs of anterior transverse commissural vessels (*atc*). Anterior commissures connecting the ventro-lateral (Figs. 2, 5 *v*) with the dorso-lateral vessels (*d*) of the hind-body, were not observed. The ventro-lateral vessels are relatively wider and the parenchymal tissue contiguous with the walls of the hind-body relatively thicker than in *N. van-cleavei.* Globules of dense excretory fluid (Fig. 5, *e*) occur in the vessels of the hind-body, as in *N. van-cleavei.* These were designated by Faust (1917a: 4) as glands with "glandular nuclei and hyaline cytoplasm." The arrangement of the vessels of the reserve bladder, in the fore-body was accurately observed by Faust (1917a: 38 and Pl. IV, fig. 49).

It is possible that the specimens herein described represent a young stage in the development of *N. van-cleavei.* For the following reasons, however, we are inclined to regard them as belonging to a distinct species: (1) our specimens were collected at the same time of year and from the same locality as typical specimens of *N. van-cleavei* taken from other hosts; (2) the specimens collected by Faust were taken too early in the spring, probably, to represent recent infestations; (3) constant anatomical difference (of slight magnitude) other than size have been pointed out.

We wish to express our grateful appreciation to Professor George R. La Rue of the Zoölogy Department and Director of the Biological Station, University of Michigan, by whom this study was suggested and under whom it has been conducted. We are indebted to Dr. Charles W. Creaser of the Biological Station, University of Michigan and of the College of the City of Detroit, for aid in the identification of fishes.

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Abbreviations Used

*a*—acetabulum
*an*—anterior nerve
*at*—anterior transverse commissural vessels
*bc*—bursa copulatrix
*cc*—calcareous corpuscle
*cm*—circular muscle fibers
*d*—dorso-lateral excretory vessel of hind-body
*e*—viscous globule of excretory fluid
*el*—network of extralateral excretory vessels
*ev*—extralateral excretory vessels
*ep*—excretory pore
*fb*—fore-body
*gp*—genital pore
*hb*—hind-body
*hf*—hold-fast organ
*hg*—hold-fast gland
*hm*—cyst membrane of host origin
*i*—intra-lateral excretory vessel
*im*—inner longitudinal muscle fibers
*in*—intestinal caecum
*l*—lateral collecting excretory vessel
*m*—median dorsal excretory vessel
*me*—marginal excretory vessel
*mp*—cyst membrane of parasite origin
*mv*—median ventral excretory vessel
*o*—outer longitudinal muscle fibers
*p*—primary lateral excretory vessel
*pn*—posterior nerve
*pt*—posterior transverse commissural excretory vessel
*r*—fundaments of reproductive organs
*s*—supra-pharyngeal nerve commissure
*v*—ventro-lateral excretory vessel of hind-body

Explanation of Plate VI

All of the figures pertain to *Neascus ptychocheilus* (Faust). Fig. 1 was drawn from a living specimen not under cover-glass pressure. Figs. 4 and 5 were drawn from living specimens flattened under a cover-glass. The guide line applies to Figs. 2 and 3, which were drawn with the aid of a camera lucida.

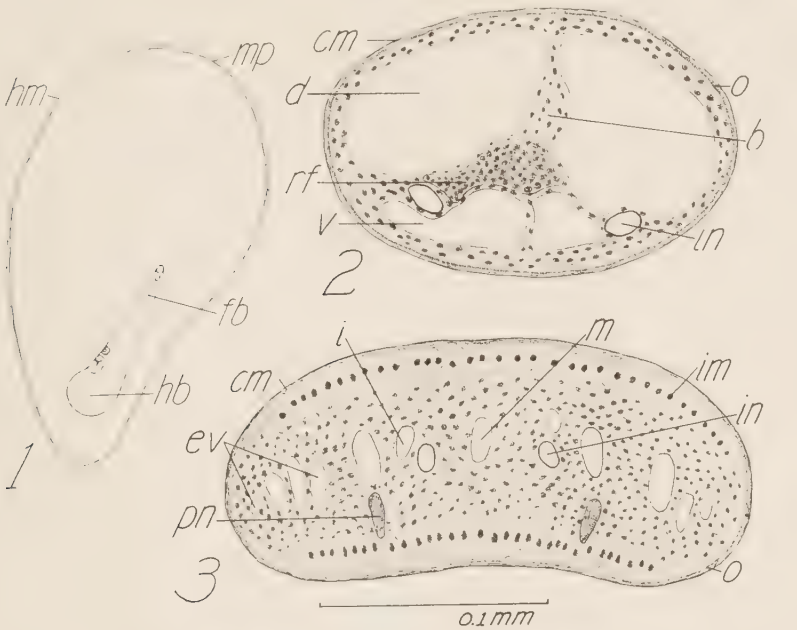
Fig. 1.—Parasite within cyst.

Fig. 2.—Cross-section of hind-body through anterior part of bursa copulatrix.

Fig. 3.—Cross-section of fore-body, anterior to acetabulum.

Fig. 4.—Ventral view showing external features and certain internal organs. Hold-fast gland and reproductive fundaments are represented as seen in mounted specimens.

Fig. 5.—Ventral view showing principal vessels of reserve bladder.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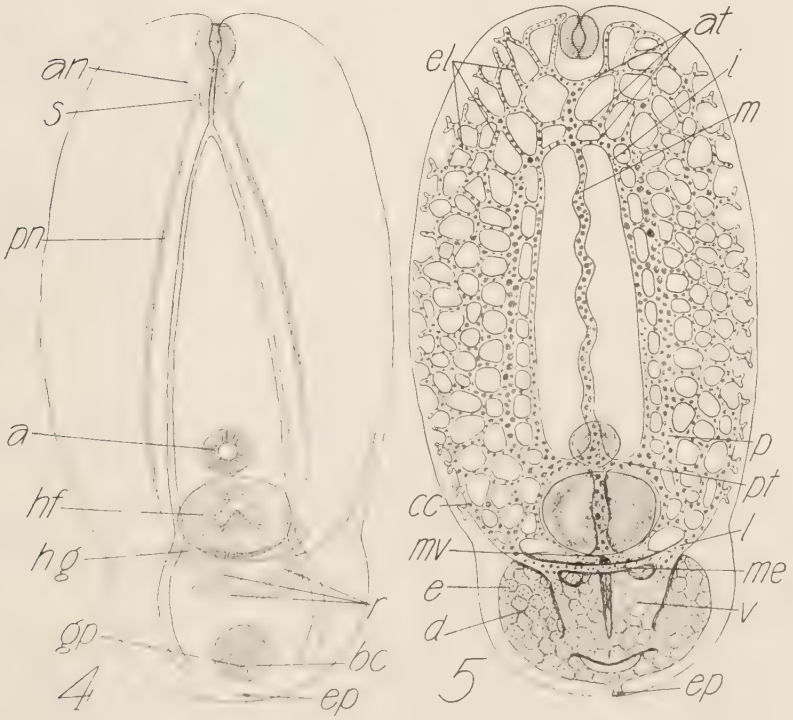
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PLATE VI

# A LINGUATULID PARASITE FROM NORTH AMERICAN FISHES

Fred J. Holl
Duke University

During the examination of fishes the writer has found an apparently new species of linguatulid living as a parasite in the liver, mesentaries, and swimbladder of the yellow bullhead, *Ameiurus natalis* (Le Sueur) and the pumpkinseed, *Eupomotis gibbosus* (Linnaeus). The fishes were collected from an artificial lake, about one hundred years old, near Gibsonville, N. C. As far as the author has been able to learn these are the first linguatulids reported as parasites of North American fish.

The taxonomic position of Linguatulidae has been a puzzle to zoologists for many years and even now there is some controversy as to the position of this group in the animal kingdom. The group has been placed with the cestodes, trematodes, nematodes, and leeches. Van Beneden was the first to recognize their arthropod nature and placed them in crustacea. Schibart suggested that they were Acarina and he was supported by Leuckart, who advanced embryological and anatomical evidence to support this view. Shipley (1898) divides the family into two genera, as suggested by Leuckart, and describes twenty-four species. Shipley (1909) adds a third genus and lists twenty-six species. Sambon (1922) divides the family into thirty genera and forty-six species. In describing these he refers to external characteristics and anatomical characters which are easily seen.

Linguatulids have been reported from North America as parasites of birds, reptiles, and mammals. Ward (1899) reported *Reighardia sternalis* (Diesing, 1864) as a parasite in the Common Tern and Bonaparte's Gull. Both hosts were collected in the Great Lakes region. Sambon (1922) records eight species as parasites of North American snakes, alligators, and mammals. Hett (1915) describes *Porocephalus globicephalus* from a single female specimen from the lungs of an American "moccasin," *Tropidontus fasciatus* (Linn). Job and Cooper (1917) give additional information concerning this species from specimens taken from the black snake *Bascanion constrictor* (Linn) collected at Garrison-on-Hudson, New York. H. W. Stunkard of New York University informed me that he found linguatulids in the lungs of turtles.

Diesing (1835) grouped a number of linguatulids from fishes collected in Brazil under the name *Pentastoma gracile* Diesing. He reported twenty species of fishes (nine in family Siluridae) as hosts of linguatulids. *Subtriquetra subtriqueta* (Diesing, 1835) Sambon, 1922, was reported from Acara coscuda from Brazil. Beauchamps (1918) records

a linguatulid parasite from fishes of the Belgian Congo. Pearse (1920) reports under the name *Porocephalus gracilis* Diesing a parasite which occurred encysted in the liver and peritoneum of *Aquidens pulcher* (Gill) collected from Lake Valencia, Venezuela. This linguatulid is similar to those found by the writer but differ in the size and proportions of the parts of the hooks.

Sambon (1922) characterizes the subfamily Porocephalinae as follows: "Female genital pore at posterior end of abdomen. Uterovagina tubular, greatly elongated and forming numerous winding. Mouth in a line with or posterior to hooks. Salivary glands greatly developed, extending whole length of body on either side of the alimentary tube. Larva with four legs." The linguatulids in this subfamily are divided into three sections: *Linguatulini,* flattened fluke-like forms; *Sebekini,* with hoods in trapezoid formation, alimentary canal dorsal and longer than body; and *Porocephalini,* with hooks arranged archwise, alimentary canal not longer than body and straight.

The species collected by the writer appear to belong to a new genus in the section Sebekini, Sambon, 1922. Sambon describes three genera: *Sebekia,* cephalothorax wedge-shaped and projects nipple-like from the abdomen, hooks small and single; *Alofia* Giglioli, 1922, cephalothorax continuous with abdomen, hooks are comparatively large, single, equal, and smooth; *Leiperia* Sambon 1922, body cylindrical and more or less coiled, hooks trapezoid in position, single, equal, and smooth. But the specimens I have do not fall in any one of these, hence I establish for them

BDUKUS NEW GENUS

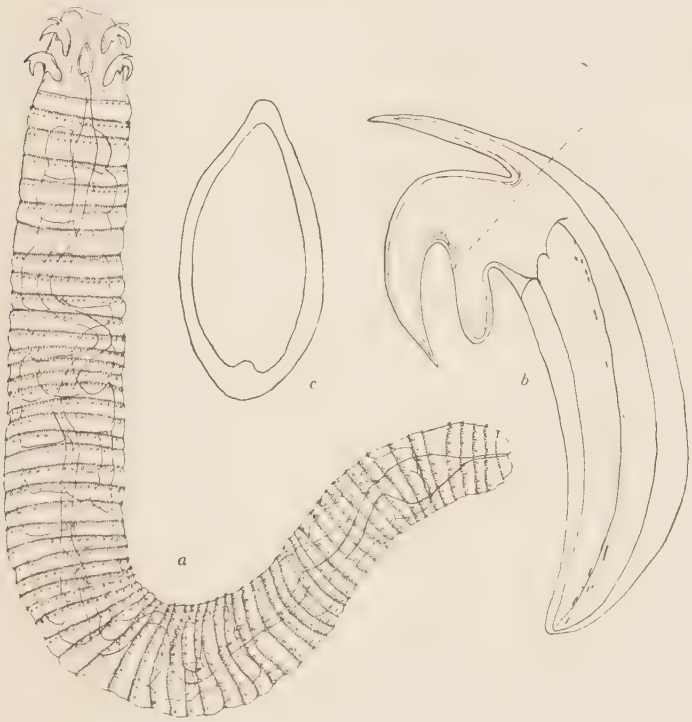
Section Sebekini: Body cylindrical with close annulations, cephalothorax about same diameter as the abdomen and continuous with it. Mouth, between hooks, oval in shape, posterior margin on line with hooks. Alimentary tract longer than body. Anus terminal. Hooks large with accessory spines. Utero-vagina long and convoluted.

Type: *Bdukus ichthyius* from *Ameiurus natalis* (Le Sueur) and *Eupomotis gibbosus* (Linnaeus) collected at Gibsonville, North Carolina.

*Bdukus ichthyius* n. sp.

Specific diagnosis: *Bdukus:* Female: length, 4 mm.; width at middle of body, 0.46 mm.; Cephalothorax length, 0.32 mm.; width opposite posterior pair of hooks, 0.33 mm. The mouth is on the ventral side between the bases of the hooks and is oval in shape surrounded by a wall of chitin; length, 0.11 mm.; width, 0.03 mm. (text figure C). The mouth opens into the esophagus which grows gradually wider up to the third abdominal segment. The diameter of the enteron becomes less and continues about the same size until near the posterior end,

where it increases in diameter and forms the fusiform rectum. The outline and diameter of the enteron can be easily seen in specimens filled with food. The hooks are hollow and arranged in trapezoid formation, lateral to the mouth and inserted in pockets which extend about 0.13 mm. below the cuticula. One pair of hooks are inserted anterior to the mouth and the other pair at about the level of the posterior margin of the mouth. Each hook has an accessory spine and a radicular process. A row of spines extend around each segment at the posterior margin and




EXPLANATION OF TEXT FIGURE

All figures represent *Bdukus ichthyius: a,* entire specimen, × 112; *b,* hook; the broken line shows the region outside the body wall, × 325; *c,* mouth, × 325.

overlaps the anterior end of the segment behind. A series of thirty to forty pores, the openings of epithelial glands, form a circle around the anterior end of each segment. The anus is terminal and the genital pore is on the ventral side immediately anterior to the anus. The female specimens studied measured from 3.5 to 5.63 mm. in length and the males from 2.3 to 2.7 mm.

The writer wishes to thank Doctor A. S. Pearse for kindly advice and criticism in preparation of this paper.

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## HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The one hundred and eighth meeting was held December 17, 1927.

Dr. Benjamin Schwartz reported the following notes:

1. The occurrence of Taenia in the liver of the giraffe.—A post-mortem examination of a giraffe which died in the National Zoological Park, Washington, D. C., on November 26, 1927, revealed the presence of numerous cysticerci throughout the liver. Each cysticercus contains, in addition to the four suckers, a small rudimentary sucker, similar to that of *T. saginata*. The head is unarmed. In Von Linstow's Compendium the giraffe is listed as a host of *Cysticercus bovis*, the record being based on a report by Weinland (1863). Another original record of the occurrence of this larval tapeworm in the giraffe is that of Möbius (1871) who reports the parasites from the liver and states that they are identical with *Cysticercus bovis* from cattle. Dr. Schwartz stated that he fed several cysticerci obtained from the giraffe to two dogs and a cat and that these animals were killed two weeks after feeding with negative results.

2. The occurrence of larval tapeworms belonging to the genus Tetrathyridium in a baboon (*Papio porcarius*).—The baboon was received in the National Zoological Park, November 1, 1926, and died May 17, 1927. Three larval tapeworms embedded in connective tissue cysts were found to be about 2 mm. in diameter. The larva is about 1.85 mm. long; its head, which is invaginated, contains four suckers but lacks a rostellum and hooks. Larval tapeworms belonging to the genus Tetrathyridium are known from mammals, birds and reptiles. About 10 species of Tetrathyridium have been described and named and several forms have been reported merely as *Tetrathyridium sp.* One of the larvae from the baboon was fed to a cat with negative results.

3. The occurrence of larval tapeworms in the liver, lungs, spleen, kidneys, omentum and heart of the squirrel (*Sciurus carolinensis*).—A recent post-mortem examination of two squirrels, one from Bowie, Md., and the other from Falls Church, Va., revealed an intense infection with larval tapeworms in the liver, lungs and spleen and a less marked infection of the kidneys, omentum and heart. Each cysticercoid contains one or more heads. The head is armed with a double crown of very small hooks about 15$\mu$ long. Attempts to rear these tapeworms in dogs, cats, rats and a pigeon yielded negative results.

Dr. Robert Hegner reported the results of experiments with the transfer of trichomonad flagellates of the monkey. Attempts were made to infest the vagina with the intestinal form of these flagellates with partial success.

Merritt P. Sarles presented a note on the age resistance of the domestic cat to infections with the hookworm, *Ancylostoma braziliense*. Herrick shows (in a paper to be published soon), that the dog possesses a marked age resistance to infection with the dog hookworm, *Ancylostoma caninum*. As there is little or no evidence of an age resistance against hookworms in man it was considered of interest to determine what relation exists between *Ancylostoma braziliense*, found in cats, dogs, and man, and one of its hosts. Accordingly a quantitative study of experimental infestations of *Ancylostoma braziliense* in the domestic cat was made on 13 animals. Immature and adult cats were given counted numbers of the infective larvae by capsule. Diagnosis was made by the Lane technic for the first appearance of hookworm eggs in the feces, and the subsequent egg production of the worms was then studied for one month by means of dilution egg counts. At the end of that time the animals were sacrificed and the worm burden determined. A definite age resistance of the cat to this parasite was found to be present as indicated by the following observations: 1. The time required for the worms to reach sexual maturity (as indicated by the first appearance of eggs in the feces) was longer in the old animals than in the young animals. 2. The time required for the maximum egg production to be reached was longer in the old animals, apparently indicating a delayed development. 3. The percentage of the larvae

given which developed in the old animals was much smaller than the percentage developing in the young animals. The study also showed that in infestations with *A. braziliense* in cats, as in Herrick's work with *A. caninum* in dogs, a definite peak occurs in the daily egg-output of the worms, and that the eggs produced per worm increases with the age of the worms.

Dr. G. Steiner spoke on the development of an undescribed species of Hexamermis found in *Parajulus pennsylvanicus*. This Hexamermis has "missing link" characters and its development throws much light upon the phylogeny and relationship of the mermithids. (Details will be published elsewhere.)

Dr. E. W. Price reported the following items:

1. The occurrence of *Prohemistomum appendiculatum* in the United States.—On June 16, 1927, six specimens of a small trematode were collected from the small intestine of a local dog. These trematodes correspond closely to the description of *Prohemistomum appendiculatum*, a form described by Ciurea (1916) from dogs and cats in Roumania.

2. A note on *Schistosoma bomfordi* Montgomery, 1906, and *S. turkestanicum* Skrjabin, 1916.—Two of the schistosomes described from cattle, *S. bomfordi* and *S. turkestanicum*, do not appear to be congeneric with *S. haematobium*, type of the genus Schistosoma. In *S. haematobium* and related species the testicular follicles are few in number (4 to 5 in *S. haematobium*) and are situated slightly caudad of the acetabulum, and the ovary in the female is situated near the middle or in the posterior half of the body. In *S. bomfordi* and *S. turkestanicum* the testicular follicles are very small and numerous, and are situated in a double row extending from a short distance caudad of the acetabulum to near the middle of the body; the ovary in the female is situated in the anterior third of the body. These characters relate these species to the genus Ornithobilharzia Odhner, 1912, and it is, therefore, proposed that *S. bomfordi* and *S. turkestanicum* be reallocated to this genus.

Dr. C. W. Stiles reported a case of *Phthirius pubis* on the back of the hand. A young man came to the laboratory in some consternation. He had been working in a laboratory which studies communicable diseases and he feared that an ectoparasite from one of the experimental animals had gotten on him and might infect him with one of the diseases under experimentation.

Miss L. Reardon reported a possible but somewhat doubtful case of *Gordius aquaticus* in an infant. Fragments of a worm recently sent for determination proved to be a male *Gordius aquaticus*. A child in East Hartford, Conn., patient of Dr. Ernest Caulfield, Hartford, Conn., September, 1927, exhibited abdominal pains and some vomiting which cleared within 24 hours. About 2 months later the mother discovered these fragments in the bowl of the toilet after the child had used the closet. In the interval, September-November, the child did not exhibit symptoms which were referable to verminous infection. Thus, the evidence is not complete that this is actually an instance of infection with Gordius but the possibility of infection is not excluded. It is interesting to note that the East Hartford district where the patient resides was recently flooded in the Connecticut River flood but there seems to be no evidence that the public reservoir supply was directly affected. The possibility is, of course, present that the worm was originally in the reservoir and that it passed through the water pipes to the house. The incident is considered of interest from two viewpoints: first, if the Gordius actually came from the child, this represents an additional case of a very rare infection by this worm; secondly, if the worm did not actually come from the child it represents an interesting case of the transportation of the Gordius for at least a considerable distance through a public water supply.

Dr. N. A. Cobb reported on diseased Narcissus bulbs from France. In the Official Record of the Department of Agriculture, May 26, 1926, there was published a description of a new disease of narcissus bulbs caused by the nema *Aphelenchus subtenuis*, there described for the first time. The symptoms of this disease so closely resemble those caused by *Tylenchus dipsaci*, the well known

stem nema, as to make it difficult, if not impossible, to tell the two species apart on the basis of the symptoms. The distribution of the disease in the United States was given so far as then known, and it was made apparent that the disease had been recently imported from Europe and that apparently it must be indigenous there. A recent shipment of narcissus bulbs from Carqueiranne, on the Mediterranean, east of Marseilles, contains bulbs carrying this disease, this being the first occasion on which it has been definitely shown to exist in Europe. Apparently there can be no question, not only that it exists in Europe, but that it must be widespread. This opinion is based on the occurrence of the disease in American plantings of bulbs derived from widely different sources in Europe.

The one hundred and ninth meeting was held January 21, 1928.

The following resolution was adopted:

The Helminthological Society learns with profound regret of the death of Professor Francesco Saverio Monticelli, a foreign corresponding member of this society since 1911. His election among the first group of twenty foreign parasitologists was a recognition of his distinguished achievements, now extending over forty years. He has joined the illustrious group of Blanchard, Ijima, Linstow, Looss, Luehe, Manson, Parona and Shipley, elected at the same time and now passed away. His work remains as the scientist's most fitting memorial and will long perpetuate his memory. This society laments his passing while at the same time it pays tribute to his worth and accomplishments. No better monument could a man have than that posterity remember his as a life well spent in the advancement of human knowledge.

Dr. Benjamin Schwartz was elected the Society's representative in the Washington Academy of Sciences.

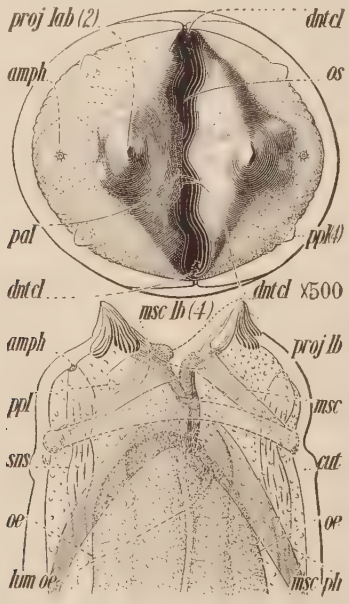
Dr. N. A. Cobb presented three notes as follows:

1. The Fossores of Syringolaimus.—This calls attention to a new species of the nemic genus Syringolaimus, a species found, among other places, in the algal "incrustations" on the marine snail, *Alectrion obsoleta*. This snail lives between tide marks, so that twice a day it is exposed to the air, and hence the habitat on its surface is peculiar;—among other things, in its rapidly variable temperature and salinity. The peculiarity of this habitat could readily be thought to account for the interesting peculiarities of the genus Syringolaimus. The "mandibles" of this new syringolaim, three in number, have an outward throw, and have been seen in action when the nema was placed under the microscope under slight pressure. Their motion is through an angle of about 180°, and under the circumstances mentioned takes place every second or so. The nema feeds, at least at times, exclusively on a microscopic alga, a species of Ralfsia(?). The contents of the Ralfsia cells are highly characteristic and it was possible to identify them in the contents of the intestine of the syringolaim. As the Ralfsia is covered with a rather impenetrable coating of filamentous green algae of other microscopic species, it can be reached by the syringolaim *only by digging*. These various circumstances make it clear that in this case the reversed "mandibles" are veritable digging organs. The word "fossor" is proposed for use in connection with such organs. Fossor: (plural, fossores; collective, fossorium); a tool or organ used for digging, usually existing in a plurality, and acting symmetrically outward from a plane or axis. Related to "fossorial,"—said of animals that dig.

2. Nematized teasel seed.—Attention is called to what appears to be the first observed case in the United States of the nemic infestation of the seeds of teasel, *Dipsacus fullorum*. This parasitism is historically interesting inasmuch as teasel was the first plant in which the nema *Tylenchus dipsaci* was discovered. There is good reason to suppose that in the present case the disease was recently imported into the United States on teasel seed. Experiments carried out in Agricultural Technology, aided by the Seed Laboratories, Department of Agriculture, show the teasel *seed* to be infested by the nema, sometimes 50 or more nemas to an infested seed. Hot water treatment of the seed at 118°F. for a half hour denema-

tises the seed while reducing the viability to from 80 to 85% if they be sown while yet more or less moist, so that the application of hot water becomes in this case also a practicable means of combatting this pest.

3. The amphids of the nema *Physaloptera phrynosoma.*—The literature of this genus indicates uncertainty as to the number of cephalic papillae, opinion being divided between the numbers 4 and 6. This discrepancy may be accounted for, at least in *P. phrynosoma,* by the fact that there are four papillae and two amphids situated practically in the same circlet. While the papillae are slightly elevated, the amphids are slightly depressed, and, as usual, it is possible to distinguish these two kinds of organs from each other by their innervation, the innervation of the papillae being single while that of the amphids is multiple. Some specimens showed a spiral structure (or attitude) in the external amphids, this being the first occasion, I think, on which spirality has been observed in the external amphids




Text fig.—*Physaloptera phrynosoma.* Front and ventral views of the head, showing the two frontal labial projections, the amphids and the four submedian papillae. In the ventral view the sensillas are shown, *sns.* In both figures a sort of denticulate palate is shown on the right jaw, *pal* and *dnt. cl.* The muscles and framework for opening and closing the jaws are also shown;—labial muscles, four in number, *msc lb,* and the pharyngeal muscles, *msc ph.*

of a parasitic nema. It is now well established that the spiral is a fundamental form of the external amphids of the free-living nemas. The relationships are set forth in the accompanying figure, whose lettering is self-explanatory.

Dr. C. W. Stiles and Mrs. Mabelle Orleman Nolan exhibited a specimen of *Cysticercus cellulosae* from the human brain, sent in by Dr. G. B. Kramer, Director of Laboratories of the Youngstown, Ohio, Hospital Association. Patient was a male, about 50 years old, working in one of the steel mills. He was taken to the hospital in convulsions which were practically continuous and, of course, the patient was unconscious. He died four hours after admission. There were no relatives to give a definite history. All that is known of the man is that he was

a foreigner and worked in the steel mills. Autopsy was performed by the coroner and Doctor Kramer was present by request. Twelve cysts were found scattered in various parts of the brain, all of them about the same size and appearance as the one exhibited and each cyst was attached to the brain tissue by a delicate pedicle. No cyst was found in any other organ of the body. The specimen is U. S. Public Health Service No. 12333.

Dr. G. Steiner reported on some experiments regarding the transmission of so-called virus diseases of plants through soil and plant-parasitic nemas. The agency of experiments so far seem to establish the fact that the root-knot nema (*Caconema radicicola*) is able to transmit tomato mosaic.

O. R. McCoy discussed the life history of *Echinoparyphium flexum* (Linton 1892). [To be published elsewhere.]

J. R. Christie presented two notes as follows:

1. Experiments with *Agamermis decaudata* have demonstrated this to be a strictly bisexual species, incapable of producing viable eggs without fertilization by a male. (Details to be published elsewhere.)

2. *Ceratomegilla fuscilabris*, a new host for *Agamermis decaudata*. Several years ago the Coccinellid beetle, *Ceratomegilla fuscilabris*, occurring in certain localities near Falls Church, Virginia, was found to harbor a mermithid parasite. The mermitized beetles all came from localities where the grasshoppers were infected with *Agamermis decaudata* and larvae dissected from the beetles showed no morphological characters which served to distinguish them from this parasite. However, the finding of a mermithid which had previously been considered typically, if not exclusively an Orthopterous parasite, the females of which normally attain a length of from 200 to 400 mm. in a small beetle not over 5 or 6 mm. long invited further investigation. What would happen if an *A. decaudata* preparasitic larva of female potentialities (provided they have sex potentialities) entered one of these beetles or indeed if several such larvae entered? On one occasion ten larvae were dissected from a single host. During the summers of 1926 and 1927 a total of 14 mermithids were bred from the beetles, all of which, on reaching maturity proved to be males, and a plan to study eggs and preparasitic larvae from parents, both beetle reared, was of necessity, abandoned. Certain observations which will be published elsewhere, lead me to believe that larvae which enter these beetles never develop into females. Three virgin *A. decaudata* females bred from grasshoppers were placed, each in a separate container of soil and with each was placed *a male bred from C. fuscilabris*. Typical *A. decaudata* eggs were produced in each case. The preparasitic larvae which developed from these eggs were entirely typical of this species as were also the parasitic larvae resulting when grasshopper nymphs were infested. It would seem that the mermithid occurring in these beetles is *A. decaudata*, and *C. fuscilabris* must be added to the list of natural hosts for this species.

The one hundred and tenth meeting was held on February 18, 1928.

Dr. Armando Rodriguez Caceres of Cuba was elected to membership.

Dr. N. A. Cobb presented a description and drawings, both prepared by Mr. Gerald Thorne, of what apparently is a new species of Heterodera found on wheat in Saskatchewan, Canada. Mr. Thorne's proposed new species differs from *Heterodera schachtii* in the following points:—The cuticula is punctate. The female body lacks the caudal projection. The females are much smaller, averaging 0.52 mm. in length and containing only 78 eggs as compared to the average length of 0.8 mm. of *H. schachtii* and 280 egg content. The life history closely follows that of *H. schachtii*. It has been found in fields broken from prairie sod only four or five years ago which indicates, in Mr. Thorne's opinion, that it is no doubt originally a parasite of some indigenous plant, probably one or more of the grasses. (Details will be published elsewhere.)

Dr. Cobb exhibited a photograph and a drawing of the spicula of a new species of Spironoura, the latter having been prepared after treatment with potassium hydrate, and called attention to a certain extensive wing-like structure which was

visable only after such treatment. In this connection the structure and significance of the telamon was discussed by Dr. Hall. Dr. Cobb also exhibited photographs of optical cross sections of nematode heads as seen in cephalic aspect and disclosed the possibilities and limitations of such photographs.

On an inquiry by Dr. Steiner regarding the possible effectiveness of naphthaline as an anthelmintic Dr. Hall pointed out that in his experience with this substance it had shown little promise.

Dr. E. W. Price reported a case of spurious parasitism in man and exhibited specimens of the supposed parasite. The objects, which appear to be undigested milk coagula, were forwarded to the Bureau of Animal Industry for identification with the statement that similar bodies had been passed by the patient for about 30 years, and were suspected by the attendant of being segments of a tapeworm, *Diplogonoporus grandis*. The symptoms given were diarrhea, severe "hives," diminished appetite and gradual loss in weight, anemia, aching of body, and pricking sensation of the rectum. Various anthelmintics had been tried without success.

J. R. Christie presented the results of recent experiments regarding the effect of environment on the development of sex in the Mermithidae. Referring to *Mermis subnigrescens* he pointed out that when the host became heavily infected the resulting parasites were males; when lightly infected, females. (Details will be published elsewhere.)

Dr. J. H. Sandground presented a demonstration showing specimens of *Ternidens deminutus* received from Dr. W. L. Thomson who had recovered them from stools of natives in Southern Rhodesia after anthelmintic treatment. Eggs and infective larvae probably referable to this little known parasite were also exhibited, the material having been secured from an infection in a missionary coming from the same locality from which Dr. Thomson's material had been secured. The possibility of infection with Ternidens being confused with human hookworm infections and of its being a more common parasite of man than the few references in the literature might indicate was suggested.

A note on parasites of rats (*Rattus norvegicus* and *R. norvegicus albus*) was presented by Dr. E. B. Cram:—In wild rats (*Rattus norvegicus*) that were killed in the grounds of the National Zoological Park, Washington, D. C., several years ago, the writer found heavy infestations of the stomach with a spirurid which she subsequently described as a new species, *Protospirura columbiana*. From these same rats there were also the following findings which are thought of sufficient interest to be put on record. *Hepaticola hepatica* was found in the livers of three rats, a spotty appearance of the liver being caused by large numbers of the eggs; the outline of the worms was also evident in the liver tissue and in one case a long filariform nematode was found, lying between the liver and the stomach, and was later identified as a female specimen of *H. hepatica*. Masses of eggs were "glued" to the outer surface of the worm, and others were present within the uterus, all corresponding to those found deposited in the liver of the rats, and corresponding to *H. hepatica* as distinct from the two later species, *H. gastrica* and *H. soricicola*. Complete specimens of *H. hepatica* formerly have had to be dissected from the liver, and, being very delicate, were obtained only with great difficulty; the reason for the spontaneous desertion of the liver by the specimen discussed above is unknown, although the fact that the viscera of the rat were rather badly decomposed may be partly responsible. A second finding of interest in one of these rats was that of a fluke, in the washings of the abdominal viscera; this fluke has been identified as *Amphimerus speciosus* (Stiles and Hassall, 1896) Barker, 1911, which has formerly been known only from birds. Stiles and Hassall in 1894 described a fluke, found in the biliary ducts of crows (*Corvus americanus* and *C. ossifragus*) in the District of Columbia and Maryland, as *Distomum longissimum* var. *corvinum* and two years later, having compared their form with specimens of Linstow's *D. longissimum* and having concluded that the two were distinct, made of theirs a new species, *Opisthorchis speciosus*. This parasite was also found and identified in 1906 by Graybill in a great white egret at the National

Zoological Park, the same locality as the present finding in the rat. The only fluke of the family Opisthorchiidae which has formerly been found in rats appears to be *Clonorchis sinensis;* that natural infestations of this form have been found and experimental infestations have been produced frequently, indicates that the rat is a suitable host for this group of flukes, and the present record of a species known until now only in birds is therefore not unparalleled. In addition to these findings in wild rats, postmortem examinations conducted within a period of a few weeks on 113 white rats (*Rattus norvegicus albus*) raised for laboratory purposes, showed the following cases of parasitism: (a) only one instance of a spirurid (*Protospirura muris*), and that of a single specimen, in the stomach; (b) a total of 26 cases (23% of the rats) harboring tapeworms (*Hymenolepis nana*) in the small intestine, the number of cestodes varying from 1 to 556, with an average of 48 specimens for each infested rat; (c) a total of 27 cases (24% of the rats) harboring *Syphacia obvelata* in the large intestine, the number of nematodes ranging from 3 to 926, or an average of 101 specimens for each infested rat; (d) a total of 24 cases (21% of the rats) harboring *Trichosomoides crassicauda* in the urinary bladder, the number of these nematodes varying from 1 to 7, or an average of 2.4 specimens for each infested rat. This series furnishes data as to the helminths present in a certain group of white rats all raised under the same general conditions; the number and the nature of the parasites would undoubtedly vary among rats from this same breeding laboratory as conditions might change, or among rats from other breeding laboratories.

Dr. Benjamin Schwartz called attention to the fact that the host of *Ostertagia houdemeri* described by him from the stomach of an unknown member of the Cervidae in The Journal of Parasitology, v. 13, no. 1, pages 25 to 28, is *Cervulus muntjac,* this host name having been furnished by Professor E. Houdemer who collected these nematodes in French Indo-China.

J. R. Christie, *Secretary.*

# Book Reviews

ZEITSCHRIFT FÜR PARASITENKUNDE. Abteilung F, Zeitschrift für wissenschaftliche Biologie. Berlin, 1 Bd., 1 Hft. Verlag von Julius Springer, 1928.

The appearance of a good journal in the field of parasitology is sure to be welcomed by workers and its publication under such favorable auspices is assurance of the high standards which will govern the character of the new undertaking. Edited by Professor A. Hase of Berlin with the collaboration of eleven colleagues in Germany and Austria, the first number indicates clearly the breadth and strength of the publication. The journal plans to include zoological and botanical papers in the field of general and special parasitology. It will embrace both more extended contributions and also briefer papers although none of the latter are included in this first number. In that one finds seven papers covering in all 230 pages of text with 69 text figures and one double plate. The first article by H. Graf Vitzthum covers a series of studies on the nomenclature and biology of forms in the genus Acarus including many new observations together with the older material and furnishes interesting data on the mutual struggle between parasite and host. The second article by Harald Richter deals with those wood inhabiting Nectaris which are the cause of canker and which represent individual forms in a group largely composed of harmless saprophytes. The third article by Albrecht Hase is a contribution to experimental parasitology in which the author records and discusses new observations on the bite of thousand legged worms (Chilopoda). The relation between the host and the bite beginning with the accompanying pain, the start of the dermal reaction and its progress to the finish are described in detail. These important relations are of interest not only to dermatologists but to students of parasitology in general and carry further the observations on histological modifications of the skin in Epizoonoses which have been studied by Pavlovsky and others. An investigation of the Cercospora leaf-spot of the sugar beet is reported next by E. W. Schmidt and contains much new biological material on this group of plant parasites. The spores of the canker producing Nectrias is discussed in great detail by H. W. Wollenweber. The author gives data for the differentiation of some thirty species or varieties and includes an analytical key for their rapid determination. In a series of investigations on the influence of parasites on the host, G. G. Smirnow and M. Th. Glasunow present next a study on the changes in the blood of the guinea pig after primary and repeated infection with Ascaris. They describe regular morphological changes in the constituent elements of the blood and consequent relations to anemia which depends on changes in the hemoglobin and the red cells whereas the changes in the leukocytes must be regarded as the result of a toxemia. In repeated infection the majority of the larvae become encysted in the liver which functions thus as a barrier to further wandering. A final article in the number by Karl Böning presents a study of the processes of infection in virus diseases of plants. This brief review of its contents shows that the new journal gives promise of filling a real need and exercising an important influence in the development of the broad field of general parasitology.

## NECROLOGY

In the death of Dr. H. Noguchi the world has suffered a great loss. It would be impossible to add anything new to the many tributes paid to his memory by men in every field of work. The Journal desires here to record its tribute to his unselfish devotion to the welfare of mankind and his able and effective warfare against the deadliest enemies of the human race. The Japanese may well be proud of the fact that he was a member of their race but in his splendid scientific achievements he transcended the limits of a nation and contributed his services to the entire world.